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SAMPLE PROCESSING SYSTEMS AND METHODS OF SAMPLE PROCESSING

FIELD OF INVENTION

This application relates to the field of sample processing systems and methods of processing samples. The present invention may be directed to the automated processing, treatment, or even staining of samples arranged on carriers, such as slides, and in some embodiments, directed to the continuous or batch processing of samples and carriers, as well as washing elements of a sampling system. Embodiments may further relate to control systems for sample processing and data acquisition, data maintenance, and data retrieval for sample processing. Applications to which the present invention may especially relate include immunohistochemistry, in-situ hybridization, fluorescent in-situ hybridization, special staining, and cytology, as well as potentially other chemical and biological applications.

BACKGROUND OF INVENTION

Sample processing in immunohistochemical (IHC) applications and in other chemical and biological analyses may require one or a number of various processing sequences or protocols as part of an analysis of one or more samples. The sample processing sequences or protocols may be defined by the individual or organization requesting an analysis, such as a pathologist or histologist of a hospital, and may be further defined by the dictates of a particular analysis to be performed.

In preparation for sample analysis, a biological sample may be acquired by known sample acquisition techniques and may comprise, for example in IHC applications, tissues generally or even in some applications one or a plurality of isolated cells, such as in microarray samples, and may be presented on a sample carrier such as a microscope slide. Furthermore, the sample may be presented on the carrier variously and potentially in some form of preservation. As one example, a sample such as a layer or slice of skin may be

preserved in formaldehyde and presented on a carrier with one or more paraffin or other chemical layers infiltrating the sample.

Immunologic applications, for example, may require processing sequences or protocols that comprise steps such as deparaffinization, target retrieval, and staining, especially for in-situ hybridization (ISH) techniques. Previously, in some applications, these steps may have been performed manually, potentially creating a time-intensive protocol and necessitating personnel to be actively involved in the sample processing. Attempts have been made to automate sample processing to address the need for expedient sample processing and a less manually burdensome operation. However, such previous efforts may have not fully addressed the needs for an automated sample processing system. Previous efforts to automate sample processing may be deficient in several aspects that prevent more robust automated sample processing, such as: the lack of sufficient computer control and monitoring of sample processing; the lack of information sharing for processing protocol and processing status, especially for individual samples; the lack of diagnostic capabilities; and the lack of real-time or adaptive capabilities for multiple sample batch processing.

Past efforts at automated sample processing for samples presented on carriers such as slides, such as US Patent No. 6352861 to Ventana Medical Systems, Inc. and US Patent No. 5839091 to LabVision Corporation, have not afforded the various advantages and other combinations of features as presented herein.

SUMMARY OF INVENTION

Sample processing can be accomplished as disclosed herein and as disclosed by various focused designs each included in Exhibit A attached, and each hereby incorporated by reference into this present disclosure.

The disclosure incorporated by reference, such as the various examples provided of sample processing and other disclosed techniques, are not meant to limit the present invention to any particular embodiment, whether apparatus, method, or otherwise. These

descriptions are provided rather to describe various sample processing techniques in a manner in which the present invention can be understood. The descriptions incorporated by reference and the various examples should not be construed to limit the present invention to only such techniques. This disclosure, however, may be understood to incorporate the various techniques in the context of the various embodiments of the present invention.

The techniques and systems of sample processing are addressed in a fashion that may provide the processing of one or more batches of samples and carriers with common protocols or of a plurality of groups of one or more samples and carriers having differing processing protocols, such as batch processing provided in sequential or non-sequential fashion. Processing of samples may be determined by the protocol to be followed for each sample or a protocol for multiple samples. Aspects of the present invention may be especially applicable to sample processing having one or a plurality of processing steps to be performed on one, a portion, or an entirety of samples, such protocols identified in some instances by the individual carriers presenting the samples. Aspects of the present invention may be especially applicable to immunohistochemistry (IHC) techniques, as well as in-situ hybridization (ISH) and fluorescent in-situ hybridization (FISH), special staining of samples, and microarrays; especially techniques incorporating target retrieval or the staining of samples. Furthermore, embodiments may be especially directed to processing sequences addressing issues of processing control, instrument cleaning, and waste.

Embodiments of the invention may further relate to automated control systems for sample processing. Embodiments may also be directed to data acquisition, data maintenance, data retrieval for sample processing, especially information sharing of processing protocol and processing status, such as for individual samples or multiple batch processing, sample diagnostic features, and real-time or adaptive capabilities for multiple batch processing.

To achieve the foregoing and other objects and in accordance with the purposes of the present invention, as broadly embodied and described herein, the present invention may be characterized in various claims. Some of these are set forth as follows. As to other aspects of the invention claims appear in other portions of the disclosure. None of these should be

understood as limiting; other and additional claims will be presented when appropriate and no rights are waived. Further, all claims presented at any time are incorporated in the specification to afford all opportunities of presentation. Claims potentially to be pursued for some of the initially presented aspects of the invention may include any aspects described.

To achieve the foregoing and other objects of invention, and as may be further disclosed and claimed throughout this description, the invention may comprise an automated sample processing system comprising a plurality of drawers, a plurality of sample carrier retainment assemblies each removably configured with one of the drawers, and an adaptive sample processing control system to which the drawers and the sample carrier retainment assemblies are responsive. The sample carrier retainment assemblies may comprise slide retainment assembly and may be removably configured with the drawers to provide sample processing with the drawers of the system. The adaptive sample processing control system may automate the sample processing system such that one or more batches of samples may be processed according to one or more protocols, potentially indicated by information on the slides that may be automatically identified by the adaptive sample processing control system. Sample processing may comprise one or more sampling protocols and steps, such as deparaffinization, target retrieval, and staining.

A sensor may be provided in some embodiments that may automatically identify information from one or more slides. In some embodiments, protocol information may be provided by the adaptive sample processing control system. The sample processing system may process one or more slides, or one or more batches of slides, concurrently, sequentially, or in any other temporal fashion, potentially in accordance with protocol information provided by a slide having a sample or provided by the adaptive sample processing control system. Sample batches or individual slides may be inserted or removed during processing protocol steps by the control and monitoring accomplished by the adaptive sample processing control system.

Another embodiment of the present invention that may achieve the foregoing and other objects of invention may comprise a method of sample processing, comprising the steps

of: accessing at least one of a plurality of drawers, providing at least one sample carrier retainment assembly configured with at least one sample carrier, configuring at least one of the drawers with the at least one sample carrier retainment assemblies, and adaptively processing the sample carriers. The step of adaptive processing may automate the processing of samples and may allow for either or both continuous or batch processing of slides, and may afford multiple independent slide processing and in some embodiments redundant slide processing to process each slide independently.

Embodiments of the invention may further comprise a method of automated sample processing, comprising the steps of: acquiring protocol information, transmitting the protocol information to at least one sample processing system, adaptively processing samples, and acquiring sample processing information from the step of adaptively processing. Furthermore, embodiments may provide: maintaining the protocol information, maintaining the sample processing information, information sharing of protocol information, and sample processing information. These and other method steps may be provided for individual samples or multiple batch processing, sample diagnostic features, and real-time or adaptive capabilities for multiple batch processing.

Many other embodiments of the invention are disclosed and claimed in this application, some of which may comprise independently, dependently, or in combination, processing tanks, environmental control systems, sample carrier retention devices, probe arms, washing stations, mixing stations, sample carrier manipulation devices and means, sample processing probes, waste systems, probe sanitizing systems, processing material units, and various other systems, devices, apparatus, and assemblies.

Many aspects of invention are applicable to immunohistochemistry (IHC) techniques, as well as in-situ hybridization (ISH) and fluorescent in-situ hybridization (FISH) special staining of samples, and microarrays, especially techniques incorporating target retrieval or the staining of samples. Furthermore, embodiments are directed to processing sequences addressing issues of processing control, component cleaning, and waste.

BRIEF DESCRIPTION OF THE FIGURES

The accompanying figures, are incorporated in and form a part of the description, illustrate some of the preferred embodiments of the present invention. Together with the written description and disclosures of the specification, they serve to explain principles of the invention and to enable each of the disclosed embodiments.

Figure A is a depiction of an embodiment of an overall system incorporating some of the features of the invention.

Figure B is a depiction of an embodiment of a portion of a sample carrier retainment assembly of one embodiment of the invention.

Figure C is a depiction of an embodiment of a fluid source aspect of one embodiment of the invention.

Figure D is a depiction of an embodiment of a robotic movement aspect of one embodiment of the invention.

Figure E is a depiction of an embodiment of a probe processing aspect of one embodiment of the invention.

Figure F is a depiction of an embodiment of a processing tank aspect of one embodiment of the invention.

Figures 101a-c are depictions of embodiments of probe related aspects of one embodiment of the invention.

Figures 102a-b are depictions of embodiments of additional probe related aspects of one embodiment of the invention.

Figures 103a-d are depictions of embodiments of sample carrier retainment assembly aspects of one embodiment of the invention.

Figures 104a-b are depictions of embodiments of temperature control aspects of one embodiment of the invention.

Figures 105a-d are depictions of embodiments of processing tank aspects of one embodiment of the invention.

Figures 106a-c are additional depictions of embodiments of temperature control aspects of one embodiment of the invention.

Figure 107 is a flow chart of some representative process steps of an embodiment of the invention.

Figure 108 is a description of representative deparaffinization steps of an embodiment of the invention.

Figure 109 is a block diagram of an embodiment of the invention.

Figure 110 is a depiction of an embodiment of a device incorporating some of the features of the invention.

Figure 201 is a plan view of a staining apparatus according to the invention.

Figure 202 is a perspective view of a detail of the staining apparatus according to Figure 201.

Figure 203 is a perspective view of a reagent mixer according to the invention.

Figure 204 is a vertical cross-section of the reagent mixer according to figure 203.

Figure 301 is a sectional view of a staining apparatus with washing means according to a first preferred embodiment of the invention.

Figure 302 is a detailed view of the washing means.

Figures 303 to 307 are sectional views of the washing and changing of the probe in the first embodiment of the apparatus according to the invention.

Figure 308 is a schematic top-view of a staining apparatus according to a second embodiment of the invention.

Figure 309a is a perspective view of the second embodiment of staining apparatus according to the invention.

Figure 309b is a detailed view of the washing means according to this second embodiment.

Figure 310 is perspective view of an embodiment of a probe for use by the invention.

Figure 311 is a probe latch mechanism for holding the probe.

DETAILED DESCRIPTION OF PREFERRED EMBODIMENTS

The following descriptions are provided to describe various embodiments of the present invention in a manner to facilitate a more detailed understanding some of the inventive features. The variously described examples and preferred embodiments should not

be construed to limit the present invention to only the explicitly described systems, techniques, and applications. This description may further be understood to incorporate the various systems, techniques, and applications, both singularly and in various combinations consistent with the various inventive features and embodiments of the present invention. Accordingly, the following is a detailed description of a number of specific embodiments of the invention.

Figure A shows one embodiment of a sample processing system 101 in accordance with the present invention. Cabinet sections 102 form outer portions of the system and serve to address general structural considerations of the system (a top cabinet section is not shown in Figure A). The sample processing system may comprise a plurality of drawers 104 used for the handling and processing of samples and sample carriers such as slides, potentially microscope slides. Other sample carriers may be accommodated consistent with the present invention. Each drawer may be configured to accommodate sample carrier retainment assemblies, such as slide retainment assemblies, carrier racks, modules, or magazines.

One embodiment of a sample carrier retainment assembly may comprise a slide retainment assembly 106 as shown in Figure B. The slide retainment assembly may comprise a slide rack, module, or magazines. Slide retainment assembly 106 is configured to accommodate a plurality of slides (not shown) in at least one configuration in corresponding sample carrier retention devices 108. The sample carrier retainment assemblies, are utilized in the processing of samples as further described below. It should be further noted that the sample carrier retainment assembly can be removably configured with the drawers 104, and may be stackable or nested within other retainment assemblies.

One or more drawers 110 may be provided to accommodate processing materials such as reagent containers for sample processing, also further described below. A processing material retainment assembly, such as a container rack 111, shown in Figures A and E, may be utilized to accommodate reagent containers or other processing materials within each of drawers 110. Bottle inserts may be preferrably configured with the retainment assembly to

ensure proper processing material positioning within the processing material retainment assembly and the drawer.

Multiple drawers 104 allow for one or a plurality of sample processing protocols to be performed by the system 101. Past efforts at sample processing, as previously described, may have been limited to processing sequences for an entire batch of carriers within the system. The present invention, however, in part by providing a plurality of drawers and carrier retainment assemblies, may allow for multiple batch processing, including real-time or adaptive capabilities for multiple batch processing, as further described below.

Indicator elements 112 may be provided to indicate a status of the drawers and the carriers or materials within each drawer for an operator of the system. In one embodiment, visual indicators, such as light emitting diodes in preferred embodiments, indicate if a drawer is available during operation of the sample processing system, and may indicate conditions such as a locked or open condition of a corresponding drawer, carrier capacity status of the drawer or of a carrier retainment assembly within the drawer, and chemical status of the sample processing system, such as reagent loading status or capacity. A warning indication may be given by the indicator elements, as well as other indicative signals. One or a plurality of sensors may be utilized to determine the status of the drawer as indicated by the indicator elements 112 and to further provide processing status as further described below.

A processing material unit 113, shown in Figure C, may be utilized to provide various processing material to the sample processing system 101 and to afford the segregation of waste produced during sample processing and the avoidance of cross-contamination. In one embodiment of the present invention, the processing material unit 113 is configured to accommodate one or a plurality of containers 114 such as deparaffin solution or other material utilized in sample processing. In some embodiments, the unit 113 may also accommodate waste containers 116 to provide for the collection of waste material from the sample processing. Tubing or other fluid transmission elements may be connected with the containers 114 and the sample processing system 101. Tubing or other fluid transmission elements may also be connected with the waste containers 116 and the system 101.

Embodiments of the present invention may further comprise an arm 120, shown in Figure D, utilized in sample processing, potentially having robotic movement, and in some embodiments, cartesian movement. The arm 120 may comprise, in some preferred embodiments, one or more elements, such as an actuator probe 122, a syringe or probe 124, a sensor element and a non-discrete volume fluid and/or air applicator. The actuator probe may be utilized in the configuration and manipulation of the carriers in sample processing, further described below. In some preferred embodiments, the actuator probe 122 configures and manipulates the configuration of slides in the sample carrier retention devices 108 by actuation of carrier adjustment element 130 (see for example Figure B), and in some embodiments, by contact with the slides. In some embodiments, manipulation of the slides may result in a horizontal or vertical configuration of the slides to facilitate sample processing as described below.

As previously mentioned, arm 120 may comprise syringe 124. The syringe 124 may be considered a probe in some embodiments, depending upon the requirements of protocols to be performed. Syringe 124 may be fluidically connected with one or more of the following: rinse agents, such as water; containers 114, potentially removably fluidically connected for the aspiration of reagents, such as aspiration of reagents from containers and to the samples presented with the carriers; and blow off or other removal agents such as an air source. Syringe 124 may be utilized to pierce processing material containers such as reagent containers. In some embodiments, a reservoir may be provided with the arm 120 to allow for various volumes to be aspirated by the syringe 124. The unique configuration of the reservoir allows for efficient cleaning and drying of the internal portions of the syringe while allowing for the accurate pipetting or otherwise aspiration of a wide range of volumes.

In some preferred embodiments, tubing to the syringe 124 may be provided in a spiral configuration, preferably with a low volume flow path diameter. Some preferred embodiments may comprise a flow path of about 1/16 of an inch. Processing material, such as buffer or reagents, may be fluidically connected with the syringe by way of the spiral tubing for aspiration. As various agents are aspirated, the configuration of the spiral tubing

may further allow for bubble separation of various agents aspirated through the tubing. This feature allows for the prevention of agent dilution, accurate aspiration of agents, and overall aspiration integrity.

Arm 120 may, in some preferred embodiments, comprise a sensor element. The sensor element may be used to automatically determine location and other status information of components of the sample processing system, such as reagent containers, or other processing material containers, or sample carriers. In preferred embodiments, the sensor element comprises a reader or scanner, such as a CCD camera, utilized to determine status information of processing materials, such as reagents. The sensor element, for example, reads, detects, or otherwise determines information from processing material containers, such as, for example, reading a code provided on the container to determine reagent type and reagent location within the system. The sensor element may also determine status information of sample carriers. For example, in some embodiments, slides configured with a slide retainment assembly may be provided with informational indicia, such as a code, that may indicate information about the sample presented on the slide or the processing protocol to be performed. The sensor element may read the code of the slide to determine the protocol to be performed for the particular slide and sample.

A cleaning station 140 (best shown in Figures A and E and in Figures 101a to 101c cleans one or more elements of arm 120, and in preferred embodiments, may function to clean or otherwise sterilize syringe 124. In one embodiment, the cleaning station 140 may be configured to allow a drop off and pick up of elements such as syringes for cleaning while allowing the processing throughput of the sample processing system to continue. The syringe may be sterilized, for example, with a water rinse through the syringe while the syringe is positioned at the cleaning station. In other embodiments of the present invention, the cleaning station may be configured to clean or otherwise sterilize elements of arm 120, such as syringe 124, while such elements are configured with arm 120.

In some embodiments, multiple probes or syringes may be used to apply fluids required for the staining of histological tissues samples mounted or otherwise presented on

slides. The sample processing system may drop off a "dirty", contaminated, or used probe or syringe and swap it for a "clean", uncontaminated, sterilized or an unused one. One or more probes or syringes are cleaned while the system continues processing of samples, such as applying reagent with an alternate probe or syringe.

The system may access, use and wash multiple probes or syringes for pipetting or otherwise aspirating fluids required for the staining of samples mounted or otherwise presented on slides. To eliminate cross contamination a system with a single reusable probe may wash the probe between each fluid applied. The task of washing the probe can have a large impact on the throughput of the overall system. The present invention may allow for multiple probes to be available to the system for use. The system may continuously have a clean, uncontaminated, sterilized, or an unused probe available to use and sample processing is not impacted by the required cleaning routine. The cleaning routine may be necessary to eliminate the possible cross contamination of fluids and, in some embodiments, may take up to about 1 minute to accomplish. The cumulative impact of the cleaning routine on a series of processing steps can add time to the throughput capabilities of the system. The addition of multiple probes or syringes may eliminate this impact and significantly decreases the time required to process the samples.

Embodiments of the present invention may comprise a mixing station 150 (best shown in Figures A and E and Figure 102). The system may mix component fluids, such as dyes, buffers, or other processing materials, preferably on demand and as the processing steps and protocols dictate. Fluids required during the processing steps may sometimes need to be mixed with other fluids to create a final activated fluid. However, the activity levels of these mixtures can be time sensitive and may therefore only be effective for a short period of time. The on demand mixing of fluids is advantageous in that it allows the fluids to be mixed immediately before being used. The syringe or probe 124, in preferred embodiments, will aspirate fluids into and from the mixing station 150 to mix component fluids. A rinse may further be dispensed into the mixing station to sterilize the station.

As previously described, the sample processing system may, in preferred embodiments, configure the carriers, such as slides, in either a vertical or horizontal position. In some embodiments, arm 120, or in some embodiments actuator probe 122, configure and manipulate the carriers. In one embodiment, the actuator probe 122 configures and manipulates the configuration of slides in the sample carrier retention devices 108 by actuation of carrier adjustment element 130, and in some embodiments, by contact with the carriers or slides. In some embodiments, manipulation of the slides may result in a horizontal or vertical configuration of the slides to facilitate sample processing as described below. The carrier retention assembly and sample carrier retention devices may further allow the slides to be rotated independently of other slides in the slide rack.

In preferred embodiments, slides are configurable in both vertical and horizontal positions as required for the pretreatment and staining process, as shown in Figures 103. This allows for the automation of the pretreatment and staining of slides in various manners, including pretreatment and staining as accepted in conventional manual laboratory methods. The slides are initially loaded into the carrier retention assemblies, such as slide racks, and drawers in the horizontal position. The slides may be horizontally supported by adjustable carrier supports 107 (shown in Figure F). If pretreatment is required, such as deparaffinization, the system rotates the slide into the vertical position and lowers these samples into a processing tank, further described below, filled with the required fluids. In some embodiments, the slide rack is lowered to affect lowering of the slides (see Figure 103c and Figure B). To perform the staining process on the slides, as described below, the System rotates the slide to the horizontal position and a syringe or probe applies fluid to the sample, providing a horizontal staining of the sample. Each slide can be rotated independently allowing for the independent processing of different samples with different requirements.

The sample processing system may automate processing steps of samples such as histological tissue sections or cell preparations presented on slides by pre-treatment processing, such as deparaffinization. The System provides onboard pretreatment of the slides. Examples of two types of pretreatment that are usually performed are but not limited to deparaffinization and target retrieval. In some embodiments, these processes must be

performed with the slides in a vertical orientation, immersed in tanks or baths of various fluids. Deparaffinization involves immersing the slides sequentially in a series of fluids for short periods of time (potentially for about 5 or 10 minutes). The process is intended to first remove from the sample the paraffin in which it was mounted or otherwise presented, remove the paraffin solvent, and then slowly rehydrate the sample. Target retrieval, and in some embodiments epitope unmasking, involves immersing the slides in a processing tank of heated buffer, and in some embodiments, immersing for about 20 minutes, and then allowing the slides to cool for about 20 minutes. Temperature in preferred embodiments is maintained at about 95 °C. In target retrieval, a marker or other identifier is used to mark a sample portion of interest, such as a cell or structure thereof.

The system automates, and in some embodiments mimics or otherwise corresponds to the procedure and physical attributes of the supplies used manually to perform these same pre-treatment processes. Accordingly, a processing tank 170 may be provided (as best shown in Figure F and Figures 105a-d). In some embodiments, components of each processing tank 170, as shown in Figures 105, are configured within a drawer 104. In some preferred embodiments, the fluids volume needed to perform pre-treatment processes are maintained but instead of the slide orientation with each other being face-to-face, as in conventional systems, they are side-to-side, although other slide configurations are not disclaimed. The processing tanks provide even distribution of fluids across the face of the slide.

In some embodiments, the processing tanks have the ability to heat the slides. This heat is applied evenly across the face of each individual slide by a thermal device. The precision and physical application of the heat can result in standardization and repeatability of process steps. Filling and heating tasks are performed by a computer controlled scheduler, as further described below. Fluid volume may be adjusted to account for the presence or absence of any number of slides.

In some embodiments, the individual fluids used for pretreatment will be contained in the system cabinet. Deparaffinization fluids (except DI water) will be drawn into the

processing tanks, then returned to their containers for reuse. Containers are as listed for fluids one through six. On a periodic basis, the material in the "dirty" containers will be discarded. The "clean" containers will be moved up to the dirty position, and then fresh fluid added to clean position. DI water will be drawn from the large system DI water container, and discarded after each use. Target retrieval solution will be drawn from dedicated containers, and may be recycled or discarded after each use.

The system may further have the ability to vent toxic and or flammable fumes from inside the cabinet portions or enclosure to exit ports that can be connected to an external system vent or hood, such as vent 160. Embodiments may comprise exit ports from the internal enclosure of the system and exit ports from the bulk fluid containers of the system, such as reagent containers. Fumes may be isolated and removed from various compartments within the System. An environmental control system addresses the system's ventilation to ensure the evacuation of volatile organic vapors, keeping the concentration of these materials below established toxic and explosive limits.

In some embodiments, a ventilation system such as that shown in Figure 104a will draw hazardous fumes from the vicinity of each processing tank by dedicated ducts. These ducts will feed a manifold running along the rear of the instrument. A separate ventilation system for an electronic chassis of the system may be similarly isolated having independent air input and output vents. Each of these systems has independent air input and output air vents. At one end of the manifold, a centrifugal blower will exhaust the fumes to an exit port that may be connected to the facility hood/exhaust system.

The sample processing system may further have the ability to maintain and regulate the internal temperature of the system to specified temperatures. Thermal control may be needed for several heat sources within the system, as well as ambient temperature incursion into the system's internal environment. In some preferred embodiments, the internal temperature is maintained at about 24° C ±2° C. Reagents used in the sample processing system can be optimized to operate at a thermal set point for the system.

In preferred embodiments, a Peltier grid 190, shown in Figure 104b, is used to heat or cool the slides during processing of the samples. Thermal elements 192 heat the slides, in some embodiments from ambient to about 120 C in about 3 minutes.

The internal temperature of the system may be controlled, as previously mentioned in some preferred embodiments at 24°C ±2°C, through the use, in some embodiments, of one or more heat pumps, and in some preferred embodiments two thermoelectric heat pumps (heat pump 180 shown in Figures 106a-c). In preferred embodiments, each pump module has a heat sink and fan on either side of the actual thermoelectric device (TED).

Configurations of the present invention may be provided as a Peltier Temperature Control, and in preferred embodiments, one heat sink/fan pair on the inside of the system's temperature-controlled interior volume. The other heat sink/fan pair may be on the outside of the controlled volume, where it is exposed to the ambient environment of the laboratory. The TEDs themselves may be located on the boundary between the interior and exterior, where they can generate a hot side and a cold side, and move heat into or out of the instrument interior, potentially as a Peltier Effect.

In some embodiments, a CCD camera may be used to determine the position of the sample on the slide, providing for greater accuracy during sample processing.

Embodiments of the sample processing system processing system may further provide sample diagnostic capabilities. Accordingly, in some embodiments, a device may analyze samples. A camera may be used for diagnostic purposes. In some embodiments, the sample may be scanned for further analysis, potentially by computer. The camera can also be used 1) as an area locator, 2) to locate a tissue area, 3) to apply reagent based on location and area. The scanned image may be analyzed for reagent analysis or other analyses.

The processing of samples may be accomplished according to the preferred embodiments as shown in Figures 107 and 108 and consistent with features of the present

invention. Variants of these protocols and processing steps. or other processing steps, may be accomplished consistent with the present invention.

One processing sequence may broadly comprise the pre-processing of a sample, if needed, such as deparaffinization (as previously described), and further comprise target or epitope retrieval (as previously described), and sample staining.

In some embodiments, specifics of in-situ hybridization (ISH) may be addressed. Embodiments of ISH may require a small volume of agent, such as 15 microliters, to be placed on the sample. Heat control is maintained between about 95-100 C and kept constant for a period of time. Temperature is then lowered in a controlled manner.

One issue resulting from this process is that of evaporation of the agent during heating. One feature of the present invention is to provide liquid cover slipping, such as oil on top of the applied reagent, to avoid evaporative effects. Another embodiment of the present invention may provide a cover or clip over the slide, providing for capillary action to draw agents, such as reagents, over the sample while the clip or cover reduces evaporative effects.

Furthermore, microarray samples may be accommodated for processing consistent with the present invention. Very small samples may be presented on a carrier or slide, potentially referred to as "spotting". The samples may be introduced by a probe comprised of the sample material to be presented on the slide or of various other materials.

Special stains may also be accommodated by the present invention individually and in accordance with processing such as IHC or ISH. IHC and ISH staining may typically be performed with an antibody agent. Special stains, however, may be comprised of dyes or metal precipitates for sample identification, such as to physically color portions of the sample. Special stains typically require larger volumes. Therefore, in some embodiments a slide boat or cover may be provided to retain the larger volume of stain over the sample.

Furthermore, fluorescent staining or tagging in IHC or ISH (FISH) may be performed consistent with the features of the present invention.

The sample processing system automates the processing of samples mounted on carriers or slides. This configuration of the system allows for the flexibility for both continuous or batch processing of slides with the design lending itself to meet established laboratory workflow demands. The multiple independent and redundant slide processing subsystems found within the system may also maintain its ability to process each slide independently.

In some embodiments, the system may comprised of independent and redundant slide staining modules (some embodiments may comprise eight modules) as shown for some embodiments in Figures A and 110. Throughput is based on time to first result with the system allowing access to completed slides as soon as a staining module has completed the scheduled staining tasks. The multiple independent and redundant staining modules allow for both continuous and batch processing of slides. Additionally, each independent staining module also allows for the independent pre-treatment and staining of each slide. A carrier retainment assembly, such as a slide retainment assembly, is used to introduce slides to be processed into the drawer 104, the drawer, slide retainment assembly, and components thereof forming a stain module. The slides may occupy one or more positions of the slide retainment assembly, such as at carrier retention devices, up to the capacity of the slide retainment assembly with the potential for each slide being processed independently of other slides configured with the slide rack. Embodiments of the stain modules, drawers, slide racks, and components thereof are also shown in Figure 110. Figure 110 also provides other embodiments of system features, such as an embodiment of the arm 120 and the component features of the arm.

Slide retainment assemblies having one or more slides may be introduced into the staining modules by introduction into drawers 104 one at a time or in any combination until all staining modules are occupied. There may be no restrictions as to the order, number or timing of when the slide retainment assemblies are introduced into the system, the system

allowing for adaptive scheduling of sample loading. Staining modules, and in some embodiments the drawers of the staining modules, will lock out access to the slides during the processing period and may release them to the operator upon completion of the staining process on the last slide. In some embodiments, the order in which the slide retainment assemblies are released is dependant on the time required to process the last slide of the retainment assembly. Slides may be processed in the most time efficient manner independently of the order to which they were introduced into the system.

The control of the processing samples may be accomplished according to the following preferred embodiments, one preferred embodiment shown in Figure 109, although other processing may be accomplished consistent with the present invention.

A sample processing system manager, such as a computer server may be connected with individual sample processing systems. This may be accomplished over internet connections but more preferably is accomplished over LAN connections. Each sample processing system may be individually controlled, in some embodiments, by a PC attached with, internal to, or otherwise provided. Data sharing between sample processing systems and the system manager may be performed to allow identification, tracking, and status of sample batches, reagents, and other agents and components of the sample processing system. A determination of which system has which reagents, reagent type, slides and protocols may be performed. Log files for each processing sequence, protocol, or slide can be generated for monitoring processing status. Database maintenance (including but not limited to purge, compact, back-up, database/list functions) and system diagnostics (including but not limited to exercising active system components to verify proper operation and assisting in troubleshooting efforts) may be accomplished manually or automatically.

A control interface may be provided for the operator, such as a graphical user interface (GUI), and may accommodate various languages. Help menus may be provided to assist in sample processing. Password protection features can be provided.

Control of the sample processing may be accomplished by dynamic scheduling algorithm, and in preferred embodiments, in accordance with the continuous or batch processing previously described. The processing sequence may be controlled, in preferred embodiments, such that the various steps of a protocol for samples may be automated by one or more algorithmic controls. A preferred control may be accomplished as follows: 1) selecting a first protocol step, 2) selecting a second protocol from a restricted list of menu items that are compatible with the first protocol step, and 3) selecting subsequent protocol steps from a restricted list of menu items that are compatible with the preceding protocol step.

Furthermore, timing tolerances, referred to in some embodiments as "bubble tolerance", may be controlled as between steps, such as between aspiration cycles. Additional control may be accomplished through timing algorithms to determine time tolerances of components of the processing system, such as the monitoring of "shelf life" or viability of reagents. Furthermore, adaptive scheduling of sample and slide insertion and removal into the system, as previously described, may be accommodated on an on-going basis throughout operation of the sample processing system.

One aspect of the invention focuses on an automated staining apparatus and a method of automated treating of samples. As to this aspect, the present invention relates to an automated staining apparatus for treating samples arranged on carrier means, such as but not limited to microscope slides, located at defined positions close to or in the apparatus by removing a portion of selected reagent from a station containing a plurality of reagents and thereafter applying the reagent to a sample, e.g. a tissue, organic cells, bacteria etc., arranged on the carrier means. This aspect of the invention facilitates that two or more reagents are mixed and the mixture applied to a sample. It also relates to a method of automated treating of samples by mixing reagents and applying the mixture to the sample.

Staining apparatuses for staining and treating samples by means of probes normally comprises a first station for containing one or more reagent vials; a second station for mounting slides, a probe arranged for removing a portion of reagent from a selected reagent

vial and applying the reagent to a slide on which the sample is arranged and a drive means for moving the probe between the various stations.

US-A-5,948,359 discloses an apparatus of the above mentioned type, wherein the first station comprises a vial holder for holding 40 or more vials in order to provide a wide range of different reagents adapted for different staining purposes, and thereby the possibility of automatically staining a large number of slides requiring different staining processes. In practise it is very important that the apparatus facilitates that many different staining processes can be performed at the same time in the apparatus, because this avoids the necessity of batching slides requiring the same staining or other treatment with reagents, and processing each batch individually.

US-A-5,723,092 discloses a sample dilution well for an immunoassay analyser in which a sample and a diluent is mixed by rotating the sample dilution well. Samples such as plasma or urine are loaded into the immunoassay analyser in a number of sample containing tubes, and a probe is arranged for collecting a portion of the sample in a selected sample carrying tube and transferring the sample to the dilution well, adding a portion of diluent, such as water to the sample portion and mixing the two by rotating the dilution well, after which a portion of the diluted sample is removed from the dilution well for examination.

Mixing wells for mixing samples and diluents are also disclosed by US-A-4,323,537, in which an analysis system is described having mixing cuvettes for mixing sample and diluents by rotation and a displaceable probe for collecting the raw sample from a sample container and deliver it to the mixing cuvette, and for collecting the diluted sample from the cuvette after mixing and deliver it to a plurality of analysis stations for further, automated analysis.

An object of this respect of the present invention is to improve the known apparatuses for staining samples as well as the method for automatic staining of samples by facilitating a wider range of available processes of treatment, so as to increase the number of different staining and/or treatment processes that may be performed automatically, alternatively or additionally to provide an increased quality of some specific staining processes.

In one embodiment, this may be achieved by the staining apparatus according to the present invention, comprising a reagent mixer having a mixing cup for receiving two or more reagents and mixing means for mixing the reagents in the mixing cup, and means for applying the reagent mixture from the mixing cup to a selected carrier means.

The term staining is used for the end product of the process, by which certain parts of the sample may be stained, i.e. have obtain a different colour, either in the optic range or in another electromagnetic range, such as ultra violet, or the staining may be an detectable, preferably automatically detectable, change in properties, such as fluorescent properties, magnetic properties, electrical properties or radioactive properties. To obtain the staining, the sample normally have to undergo a series of treatment steps, such as washing, binding of reagents to the specific parts of the sample, activation of the reagents, etc. and each treatment step may include a plurality of individual treatments.

In some staining processes, it may be required for one or more treatments to use a mixture of reagents prepared from two or more separate reagents which may be somewhat incompatible e.g. unmixable, such as a water based and an oil based reagent, or insoluble, and therefore requires that the two or more reagents are manually prepared and introduced into a reagent vial shortly before starting the staining process in order to obtain the best possible staining result for the selected examination purposes. For other processes, different staining process steps require a mixture of the same two reagents but in different dissolution ratios. Some process steps requires mixtures of two or more reagents that, when mixed, have a limited time window of usability because internal chemical processes deteriorates the mixture. By providing a staining apparatus having an automated mixer integrated therein, these types of staining processes can be performed automatically instead of requiring human interaction or manual performance of some process steps in a much more automated process, and the quality of the staining process may be improved as a desired degree of mixing of reagents may be provided or an optimal application time window for a deteriorating mixture may be reached.

Thus, the present invention relates to a staining apparatus for treating samples arranged on carrier means, comprising a vial station for containing at least two reagent vials, a carrier means station arranged for intermediate storage of a plurality of carrier means, probe drive means arranged for moving a probe, wherein the probe drive means is arranged to remove a

portion of reagent from a selected reagent vial of the vial station by means of a probe and to apply reagent to a selected carrier means of the carrier means station, wherein the apparatus comprises a mixing station with a reagent mixer having a mixing cup for receiving two or more reagents and mixing means for mixing the reagents in the mixing cup, and means for applying the reagent mixture from the mixing cup to a selected carrier means of the carrier means station.

The vial station is a collection of a plurality of vials, at least two but often 20-40 vials or more, which may or may not be physically arranged in close proximity to each other. The term station does not indicate that the vials must be located within one, confined area; rather it indicates the existence of a plurality of vials. The probe drive means may be a robot arm with two or three degrees of freedom, such as an articulated arm or one track or a set of perpendicular tracks along which a probe retainer of the probe drive means may be displaced, wherein the probe retainer may be moved in a direction normal to the track or tracks. The skilled person may readily design other types of probe drive means, e.g. combinations of the above described. The carrier means may be provided to the apparatus in a two-dimensional array, e.g. constituted by individual rows of carrier means as discussed in the example below, or the carrier means may be provided in any manner known in the art, e.g. arranged in a carrousel or as a row of carrier means. The carrier means may also be arranged movably with respect to the probe drive means, such as in an endless row that is advanced automatically past the operating area of the probe drive means or as a two-dimensional array that may be moved in a direction perpendicular to a travel direction of the probe drive means, so that the probe may reach any carrier means by the combined movement of the probe and the array.

The carrier means are preferably arranged in groups or series on trays or the like, so that a plurality of carrier means may be removed from or situated in the apparatus simultaneously, and the apparatus preferably also comprises means for performing the intermediate storage of the carrier means with samples thereon and the removal of the carrier means from the apparatus automatically.

The operation of the staining apparatus will generally be controlled by means of control means, typically a computer having a central processing unit and one or more memory unit associated therewith, means for controlling the various operations of the apparatus by controlling step motors, solenoids, valves and/or other drive or control parts of the apparatus.

The control means may have one or more data communication ports for enabling data communication with external computers by wire or wireless. The control means does not have to be physically arranged within the apparatus itself but may be a computer external to the staining apparatus and connected to the apparatus via a data transmission port thereof.

It is advantageous that the probe drive means is arranged to apply the reagent mixture from the mixing cup to selected carrier means. The mixed reagents from the mixing cup may be applied to the samples by separate means, such as a separate probe and probe drive means, or the carrier with the sample in question may be moved to be situated at an outlet from the mixing cup, but a more simple solution is reached by using the probe drive means for the task.

The mixing cup may receive the two or more reagents to be mixed from a separate set of vials or other reagent sources, but it is for rationalisation of the apparatus preferable that at least some of the reagents to be mixed may come from the same vials as are used for containing reagents to be applied to the samples. In a preferred embodiment, the probe drive means is therefore arranged to remove portions of reagents from at least two selected reagent vials of the vial station and apply said portions of reagents to the mixing cup.

The mixing means of the reagent mixer may advantageously be constituted by cup drive means arranged for cyclic movement of the mixing cup, e.g. shaking or rotation in a horizontal or a vertical plane, so as to mix reagents contained in the mixing cup. The mixing of the reagent may be further improved by arranging mixing elements, such as blades or edges within the cup and stationary with respect to the cup. Other known mixer types may instead be preferred, such as shaft-driven impellers or magnetically driven impellers.

The cyclic movement is preferably a rotation of the mixing cup, advantageously about a substantially vertical axis. The rotation is in a preferred embodiment an intermittent rotation in a clockwise and in an anticlockwise direction.

In order to provide an efficient and fully automated cleansing of the mixing cup when changing from one reagent mixture to the next, it is advantageous that the mixing cup has inner walls extending upwardly and outwardly from a bottom part of the mixing cup, and the cup drive means is capable of rotating the mixing cup at an angular speed sufficient to fling

reagents contained therein out of the mixing cup. The inner walls may terminate in an upper rim at the widest inner diameter of the mixing cup, in which case the high-speed rotation, preferably about a symmetry axis of the cup, will cause the waste reagent or cleansing liquid to be flung out over this upper rim. In an alternative embodiment of the mixer, the inner walls of the cup extend upwards and inwardly above the level of the widest inner diameter, at which one or more exit openings are provided in the inner wall as outlets for the waste reagent or the cleansing liquid. The top may be open for the probe to enter the mixing cup, or it may be closed to facilitate that the cyclic movement of the mixing cup for mixing the reagents has a vertical component. The probe may in this case enter the mixing cup through one of the exit openings.

To collect the waste reagent or the cleansing liquid, it is preferred that the reagent mixer comprises a waste reagent collecting chamber having a sidewall part laterally surrounding the mixing cup and arranged to collect reagents flung out of the mixing cup by rotation of the cup.

In a further preferred embodiment, the reagent mixer further comprises a mixing cup holder for receiving and supporting the mixing cup in a releasable manner, so that different mixing cups may be used for different reagent mixtures.

In yet further preferred embodiment, replacing means are provided for replacing the probe that is moved by the probe drive means with another probe, so as to avoid contamination of the reagents in the various reagent vials by the use of more than one probe. This may be combined with a probe washing station, in which one probe is washed after it has been replaced at the probe drive means so that it is clean and ready for repeated use without risking contamination of the content of the reagent vials.

The present invention also relates to a method of fully automated treating of samples arranged on carrier means by means of a staining apparatus controlled by means of a control means, wherein the method comprises the steps of situating a plurality of carrier means intermediately in a carrier means station, each carrier means having a sample arranged thereon, applying a portion of a first reagent selected from a plurality of reagents to a mixing cup, applying a portion of a second reagent selected from a plurality of reagents to the mixing cup, mixing the reagents in the mixing cup by means of mixing means, moving a

probe to the mixing cup by means of a probe drive means, removing a portion of the mixed reagents from the mixing cup by means of the probe, moving the probe to a selected one of said carrier means, and applying the mixed reagents to the selected carrier means. so as to perform a treatment of the sample arranged on the selected carrier means.

In a preferred embodiment, the steps of applying portions of the first and the second reagents comprises the steps of moving a probe to a first, selected reagent vial by means of the probe drive means, removing a portion of the first reagent from the first reagent vial by means of the probe, moving the probe to the mixing cup by means of the probe drive means, applying the portion of the first reagent to the mixing cup, moving a probe to a second, selected reagent vial by means of the probe drive means, removing a portion of the second reagent from the second reagent vial by means of the probe, moving the probe to the mixing cup by means of the probe drive means, and applying the portion of the second reagent to the mixing cup.

In a further preferred embodiment of the present invention, the method further comprises the step of replacing the probe arranged with the probe drive means with another probe between handling two different reagents or between handling a reagent and a reagent mixture.

The present invention further relates to the use of an apparatus of the present invention as described above for exercising the method of the present invention.

The brief description of the figures relative to this aspect of the invention will now be explained in further detail with reference to the enclosed drawings, wherein

Figure 201 is a plan view of a staining apparatus according to the invention,

Figure 202 is a perspective view of a detail of the staining apparatus according to figure 201,

Figure 203 is a perspective view of a reagent mixer according to the invention, and

Figure 204 is a vertical cross-section of the reagent mixer according to figure 203.

The embodiment shown in the figures and described in details below is only an example of an apparatus in accordance with the present invention and is not limiting the wider scope of the invention as described in the enclosed claims.

A detailed description of one embodiment of this aspect of the invention involves staining apparatus 201 according to the invention is shown in figures 201 and 202. The staining apparatus 201 comprises a rectangular frame 204 surrounding a first station 202 comprising an array of compartments wherein each compartment a reagent vial 203 is placed, and a second station 205 wherein a number of separate racks 206 is placed, and where each rack comprises a number of microscope slides 207 mounted side by side in the rack 206. In the embodiment shown, each rack may hold up to 17 slides, but the rack may be designed to hold any suitable number of slides. With eight racks arranged side by side, the shown embodiments may hold up to 136 slides 207 each having a sample, e.g. a tissue mounted on the upper side of the slide, so that reagent may be applied from above to the sample on each slide.

A robot arm (not shown) for moving a probe 210 in X and Y direction as indicated by the arrows X and Y is arranged above the frame 204 of the staining apparatus. The robot arm may is therefore position the probe 210 above all reagent vials 203 as well as above all the microscope slides 207, and may further operate the probe 210 to remove portions of reagent contained in any of the vials 203, to transfer the portion of reagent and apply it to any of the slides 207 in order to provide a selected staining or treatment of the sample on each slide 207. By use of suitable control means e.g. a computer (not shown) having the appropriate software and input data for the purpose, this staining apparatus 201 is able to automatically stain or treat samples requiring different staining or treatment reagents and processes.

The same staining apparatus viewed from below the robot arm is shown in figure 202, disclosing the probe 210 being manipulated by the robot arm. The probe 210 is raised to an upper position where it is clear of the vials 203 underneath the probe 210, but the robot comprises means (not shown) for lowering the probe 210 in order to dip the probe tip 212 into the content of a selected reagent vial 203 and to suck up a selected amount of reagent for the selected staining or treatment process.

The staining apparatus 201 of the present embodiment further comprises a probe washing station 208 and a reagent mixer 209, and the robot arm is furthermore arranged to transfer the probe to the washing station 208 as well as to the reagent mixer 209. The reagent mixer 209 will be described in detail below with reference to figures 203 and 204.

The apparatus comprises a reagent mixer 209 having a mixing cup 213 wherein two or more selected reagents may be placed by means of the robot arm and the probe 210. The reagent mixer 209 thereby provides on-board mixing of any reagents contained in the reagent vials 203, and thereby more staining processes, e.g. staining requiring the use of mixing of insoluble reagents, or reagents which may only be effective a short time after mixing, are facilitated to be performed automatically within the staining apparatus without the requirement of human interaction.

The mixer 209 comprises a mixing cup 213 for receiving reagents released from the probe 210. The mixing cup 213 is placed into a holder 215 by means of a complementary snap fitting means 216 and 217 arranged on the inside of the holder 215 and the outside of the mixing cup 213, respectively. A motor 218 is arranged for rotating the holder 215 and thereby the mixing cup 213, either intermittently clockwise and anticlockwise in order to provide a mixing of reagents contained in the mixing cup, or by spinning the holder 215 and thereby the mixing cup 213 in order to fling out waste reagents or cleansing liquid from the mixing cup 213.

For the latter purpose, the mixing cup 213 is preferably provided with sidewalls 220 extending upwardly and outwardly from the bottom 219, e.g. forming a frusto-conical cavity, and the mixing cup 213 has an upper rim 214 allowing the reagents to escape from the mixing cup 213 during the spinning process.

The reagent mixer 209 furthermore comprises a housing 221 having sidewalls 222 surrounding at least the rim 214 of the mixing cup 213 and thereby forming splash faces for collecting any liquid flung out from the mixing cup 213. The housing also comprises a lid 223 for enclosing a space 224 surrounding the mixing cup 213 in order to avoid reagent spills outside the space 224. The lid 223 has a central opening 225 allowing reagents from the probe 210 to be released into the mixing cup 213 from above the reagent mixer 209 as well as allowing the probe 210 to enter the mixing cup 213 for collecting the mixed reagents.

According to a preferred embodiment the housing also comprises a hose connection 227 for draining waste reagent or cleansing liquid from the space 224, and a tap 226 is arranged for dispensing cleansing liquid into the mixing cup 213 when required.

By means of a releasable connection 212 between the probe 210 and the robot arm, it is possible to replace the probe 210 held by the robot arm by placing the probe 210 in one of a number of free washing stations 208, where it is released by the releasable connection 212, and where a new probe 10' is connected to the robot arm by means of the releasable connection 212.

Having the appropriate input data, the control means of the apparatus operates the robot arm to commence a staining or treatment run by firstly moving the probe to a first reagent vial 203, into which the probe tip 212 is inserted and liquid is aspirated up into the probe 210 in an amount corresponding to the number of samples to be stained or treated, in accordance with the input data provided to the control means. Additionally, under certain conditions, the instrument will be required to perform a reagent inventory before a staining or treatment run can commence. This inventory is accomplished by use of the probe tip 212 to actually touch the liquid surface in each reagent vial 203. To prevent cross-contamination between the reagents in the various vials 203, a cleaning of the probe 210 or at least the probe tip 212 is required after each measurement of a reagent level.

The probe 210 is subsequently, in a first operating mode moved by the robot arm towards the slide rack system 205 in which the slides 207 are mounted. The slides 207 are situated with the surface horizontally oriented and the probe 10 dispenses the required amount of reagent on the appropriate slides in accordance with the input data. Alternatively, the probe 10 is in a second operating mode moved by the robot arm towards the reagent mixer 9 where it releases the reagent into the cup 213 of the reagent mixer 209, and is subsequently moved to the probe washing station 208, where the probe 210 is released into a free washing station 208, and another probe 210' situated in another washing station 208 is connected to the robot arm. The robot arm moves the new clean probe 210' to a second selected reagent vial 203 for collecting a selected amount of reagent from the second vial 203, and the probe 210' is thereafter by means of the robot arm moved to the reagent mixer 209, where the reagent in the probe 210 is released into the cup 213 of the mixer containing

the first selected reagent. The second operating mode can according to the invention be commenced several times if more than two reagents are to be mixed for a specific staining or treatment process.

The reagent mixer 209 mixes the reagents in the cup 213 thereof, and a clean probe 210" picked up from the washing station 208 by the robot arm is lowered into the cup 213 of the reagent mixer 209 to collect the mixed reagents, where after the robot arm moves the probe 210" towards the second station 205 containing the slides 207, at which the probe 210" dispenses the required amount of mixed reagent on selected slides 207 in accordance with the input data.

The robot arm with probe 210" is subsequently directed to a free washing station 208, and the probe 210" is replaced by yet another clean probe 210"', where after the process in accordance with the first or the second operating mode may be repeated or continued with a new reagent or reagent mixture.

As to the present aspect of the invention, some of the claims potentially to be presented and for which support should be understood to exist include:

1. A staining apparatus (201) for treating samples arranged on carrier means (207), comprising a vial station (202) for containing at least two reagent vials (203), a carrier means station (205) arranged for intermediate storage of a plurality of carrier means (207), probe drive means arranged for moving a probe (210, 210', 210"), wherein the probe drive means is arranged to remove a portion of reagent from a selected reagent vial (203) of the vial station (202) by means of a probe (210) and to apply reagent to a selected carrier means (207) of the carrier means station (205), characterised in that the apparatus (201) comprises a reagent mixer (209) having a mixing cup (213) for receiving two or more reagents and mixing means for mixing the reagents in the mixing cup (213), and means (210) for applying the reagent mixture from the mixing cup (213) to a selected carrier means (207) of the carrier means station (205).

2. A staining apparatus according to claim 1, wherein the probe drive means is arranged to apply the reagent mixture from the mixing cup (213) to selected carrier means (207) of the carrier means station (205).

3. A staining apparatus according to claim 1 or 2, wherein the probe drive means is arranged to remove portions of reagents from at least two selected reagent vials (203) of the vial station (202) and apply said portions of reagents to the mixing cup (213).

4. A staining apparatus according to any of claims 1 to 3, wherein the reagent mixer (209) further comprises cup drive means (218) arranged for cyclic movement of the mixing cup (213) so as to mix reagents contained in the mixing cup (213).

5. A staining apparatus according to claim 4, wherein the cyclic movement is a rotation of the mixing cup (213).

6. A staining apparatus according to claim 5, wherein the rotation take place about a substantially vertical axis.

7. A staining apparatus according to claim 5 or 6, wherein the rotation is an intermittent rotation in a clockwise and in an anticlockwise direction.

8. A staining apparatus according to any of the preceding claims, wherein the mixing cup (213) has inner walls (220) extending upwardly and outwardly from a bottom part (219) of the mixing cup (213), and the cup drive means (218) is capable of rotating the mixing cup (213) at an angular speed sufficient to fling reagents contained therein out of the mixing cup (213).

9. A staining apparatus according to claim 8, wherein the reagent mixer (209) comprises a waste reagent collecting chamber (221) having a sidewall part (222) laterally surrounding the mixing cup (213) and arranged to collect reagents flung out of the mixing cup (213) by rotation of the cup.

10. A staining apparatus according to any of the preceding claims, wherein the reagent mixer (209) further comprises a mixing cup holder (215) for receiving and supporting the mixing cup (13) in a releasable manner.

11. A staining apparatus according to any of the preceding claims, comprising replacing means (212) for replacing the probe (210) that is moved by the probe drive means with another probe (210').

12. A method of fully automated treating of samples arranged on carrier means (207) by means of a staining apparatus (201) controlled by means of a control means, wherein the method is characterised in that it comprises the steps of situating a plurality of carrier means (207) intermediately in a carrier means station (205), each carrier means (207) having a sample arranged thereon, applying a portion of a first reagent selected from a plurality of reagents to a mixing cup (213), applying a portion of a second reagent selected from a plurality of reagents to the mixing cup (213), mixing the reagents in the mixing cup (213) by means of mixing means, moving a probe (210, 210') to the mixing cup (213) by means of a probe drive means, removing a portion of the mixed reagents from the mixing cup (213) by means of the probe (210, 210'), moving the probe (210, 210') to a selected one of said carrier means (207), and applying the mixed reagents to the selected carrier means (207), so as to perform a treatment of the sample arranged on the selected carrier means (207).

13. A method according to claim 12, wherein the steps of applying portions of the first and the second reagents comprises the steps of moving a probe (210, 210') to a first, selected reagent vial (203) by means of the probe drive means, removing a portion of the first reagent from the first reagent vial (203) by means of the probe (210, 210'), moving the probe (210, 210') to the mixing cup (213) by means of the probe drive means, applying the portion of the first reagent to the mixing cup (213), moving a probe (210, 210') to a second, selected reagent vial (203) by means of the probe drive means, removing a portion of the second reagent from the second reagent vial (203) by means of the probe (210, 210'), moving the probe (210, 210') to the mixing cup (213) by

means of the probe drive means, and applying the portion of the second reagent to the mixing cup (213).

14. A method according to claim 12 or 13, further comprising the step of replacing the probe (210, 210') arranged with the probe drive means with another probe (210, 210') between handling two different reagents or between handling a reagent and a reagent mixture.

15. Use of an apparatus according to one of claims 1 to 11 for exercising the method according to one of claims 12 to 14.

In brief, this aspect of the invention may be understood as involving, among other aspects, an automated staining apparatus and a method for treating samples arranged on carrier means is disclosed, in which portions of two or more selected reagents from a first station containing the reagents are collected individually by means of a robot arm handling a probe and dispensed in a mixing station of the apparatus. After mixing the reagents in the mixing station, e.g. by shaking or rotating the mixing cup containing the reagents, the reagent mixture is applied to selected samples by means of the robot arm and a probe.

By providing a staining apparatus having an automated mixer integrated therein, staining or treatment processes requiring a mixture of unmixable reagents, such as a water based and an oil based reagent, or insoluble reagents, can be performed automatically instead of requiring human interaction or manual performance of some process steps in a much more automated process, and the quality of the staining process may be improved as a desired degree of mixing of reagents may be provided or an optimal application time window for a deteriorating mixture may be reached.

Yet another aspect of this invention focuses on a staining apparatus and a method for washing probes. This aspect of the invention relates to a staining apparatus for manipulation of samples arranged on carrier means, comprising probe means for dispensing a portion of reagent onto a selected carrier means, means for handling said probe means, and washing means for cleaning the probe means.

This aspect of the invention also relates to a method of washing probes in a staining apparatus for manipulation of samples arranged on carrier means.

US-A-5,839,091 discloses a staining apparatus and a method of the above-mentioned kind, said apparatus comprising a wash station, wherein a probe for applying reagents onto a slide between the individual reagent applications is washed in a wash station. The wash station comprises three different receiving locations where the same probe is washed in first, second and third receiving units, respectively, in a sequential run.

By this run, the probe is cleaned so that any residues on the probe are removed, and dried before the probe is subsequently moved to the area where the reagents are stored and a selected reagent is collected from a reagent storage for treatment of the slides.

By this washing of the probe, the risk of cross-contamination of the probe is eliminated, when the probe is used for dispensing the different reagents in the staining apparatus during the staining processes of various slides with pre-selected reagents and different individual staining requirements.

This washing process is comparatively time-consuming, since no treatment of the slides occurs for as long as the probe is being washed.

In particular, when a staining apparatus instrument is loaded with slides requiring a number of different staining protocols, the probe must frequently be uncontaminated between each new reagent, which is to be dispensed. This involves time for allowing the probe to be moved to the wash station as well as time for washing the probe.

The washing of the probe takes typically about 40 seconds. This means that by a staining apparatus comprising e.g. large number of different reagents, it takes more a very long time to complete the staining protocols for the slides in the apparatus. This in turn means that the analysis of the samples on the slides is delayed accordingly.

An object of this aspect of the present invention is to provide a staining apparatus and a method for automatic staining of samples, in which the total process time for completing the staining protocol may be reduced. In particular, it is an object of this aspect of the invention to reduce the amount of time needed for cleaning the probes between the changes of reagents to be dispensed.

These objects are achieved by a staining apparatus of the initially mentioned kind wherein the probe means comprises at least two detachable probes, and where the washing means includes at least two wash stations.

According to this aspect of the invention, the probe may be detachable from the probe handling means, such as the robot arm. According to this aspect of the invention, it is realised that by utilising, the probe is detachable; washing means may be provided with associated detaching means which includes two wash stations so that a clean probe is present at one wash station when a contaminated probe is delivered to another wash station. According to this aspect of the invention, the probe handling means may simply release the first probe at one wash station and quickly pick-up a clean probe for the next reagent dispensing run. Hereby, the probe washing and the reagent dispensing processes may take place simultaneously rather than sequentially, as it is the case in the prior art. This results in a significant reduction of time for the total process run, as the delay in reagent dispensing is kept to a minimum.

Preferably, the probe handling means includes probe holding means for holding a probe; and robot means for providing a relative movement between a selected probe and the washing means, including movement of the probe handling means between the wash stations.

Preferably, the probe handling means includes dismounting means for dismounting the probe from the probe holding means. The probe handling means may preferably also include coupling means for providing a detachable, fluid sealed coupling between the probe and the probe holding means. Hereby a quick snap mounting and dismounting of the probe is achieved.

In a particular embodiment, the coupling means includes probe coupling portion on the probe holding means, which engages a corresponding receiving portion on the probe and latching means for releasably attaching the probe to the probe handling means. The coupling means may preferably further comprise a means for releasing the latching means and allow decoupling of the probe. With a latch mechanism, it is exclusively the snap mounting that is moved, the least inertia be obtained in the system.

The washing means preferably include a first wash station and the second wash station, said wash stations being identical. By having two essentially identical, it is ensured that a probe can be properly cleaned irrespective of which wash station it is deposited in.

In the preferred embodiment, the washing means comprises a nozzle head, which is mounted on a lever, which is movable between the wash stations by shifting means. The probes are washed by coupling the nozzle to the probe and blowing air or rinsing cleaning liquid through the probe. The nozzle head is common for both wash stations. Accordingly, the nozzle head preferably comprises a showerhead nozzle, said nozzle being arranged to engage the receiving portion of the probe to be washed.

The nozzle head comprises an air supply means and/or a cleaning buffer supply means to the interior and the exterior of the probe for washing the probe. The nozzle head is connected to supply hoses for the supply of air, cleaning buffer and/or other cleaning agents.

This aspect of the invention also relates to a method of washing probes, which involves the steps of delivering the first probe to a wash station of the washing means after dispensing reagent onto the carrier means, and detaching said probe from the probe handling means in said wash station.

By this method the delay is thus limited to the time it takes to transport the probe to the washing means, release the probe and couple it to a cleaned one.

In order to reduce the waiting time for the probe handling means during the washing, said means are moved away from said first wash station for selecting a clean second probe in a second wash station. The a nozzle cleaning head is preferably moved simultaneously to the first wash station and engages the first probe subsequent to its delivery in said wash station for washing said first probe.

According to a preferred embodiment, the nozzle head is shifted between the first and second wash stations for successive probe washing.

A second probe is preferably coupled to the probe handling means and moved away from the washing means for being prepared for dispensing a reagent by collecting a reagent from a predetermined reagent supply and dispensing this portion of reagent onto a predetermined carrier means

According to a preferred embodiment of a method and a staining apparatus according to this aspect of the invention, the probe is moved to the washing means after having dispensed its content of a reagent or at least a portion thereof. As explained above, the reagent from the probe is preferably dispensed to a predetermined number of slides in one specific run. This is governed by a control system. Accordingly, the control system also determines the need for dispensing another reagent. This initiates that the probe is moved to a wash station of the washing means. The probe is releasably attached to the probe handling means, which may be governed by a robotic motion system. In the washing means, the unclean probe is delivered to a first wash station, which may be configured as an elongate hose-like member. The probe is released from its attachment to the probe handling means, preferably by activating a latch mechanism. Dismounting means cooperating with the latch mechanism for releasing the probe from the probe holding means is provided. The dismounting means could for instance be a fork-shaped stationary member, which is engaged by the latch mechanism between probe holding means and probe and performs the detachment, so that the probe drops down into the receiving wash station. A nozzle head is moved from a corresponding second wash station to the first wash station, where the unclean probe is delivered after having been detached from the probe handling means. A coupling is

now performed between said nozzle head and the receiving coupling portion of the probe. The probe holding means which has just been released from first probe is moved to the second wash station where a cleaned probe has been decoupled from the nozzle cleaning head and which is ready for attachment to the probe holding means for carrying out a new reagent dispensing run. The second wash station is configured identically with the first wash station. The nozzle head arrangement is moved to a position over the first probe and a coupling is now performed between attachment means of the nozzle head and the corresponding receiving end of the probe. The probe handling means moves the clean probe from the second wash station to a predetermined reagent bottle to be used for the next process treatment of samples on the slides.

Preferably, the latch mechanism includes self-energizing device, such as a spring-loaded catch. Moreover, an active component, such as an air cylinder, is comprised in the mechanism to release the latching and allow decoupling. This active component may also retrain the probe during the detaching.

This aspect of the invention is described in more detail in the following with reference to the accompanying drawings, wherein:

Figure 301 is a sectional view of a staining apparatus with washing means according to a first preferred embodiment of the invention;

Figure 302 is a detailed view of the washing means;

Figures 302 to 307 are sectional views of the washing and changing of the probe in the first embodiment of the apparatus according to this aspect of the invention;

Figure308 is a schematic top-view of a staining apparatus according to a second embodiment this aspect of the invention;

Figure 309a is a perspective view of the second embodiment of staining apparatus according to this aspect the invention;

Figure 309b is a detailed view of the washing means according to this second embodiment;

Figure 310 is perspective view of an embodiment of a probe for use by this aspect of the invention; and

Figure 311 is a probe latch mechanism for holding the probe.

Figure 301 is a sectional view of a staining apparatus 301 comprising a rack system 324 in which a shown number of sample carrier means, preferably slides 306 are secured. The slides 306 comprise e.g. tissue samples or blood samples, and wherein a staining is to be performed for determining components, preferably for diagnostic purposes. The apparatus also comprises a section for the storage of reagent vials 318 in which the reagents required for the slides 306 are stored. A probe holder 307 shaped as a cylindrical arm and controlled by a robotic system controlling the application of the reagent for slides in a given sequence according to a predetermined staining protocol.

At the end of the free end portion of the probe holder 307, a mounting member 312 is provided for mounting a probe 302 on the probe handling means 305. The mounting means 310 between the probe handling means 305 and the probe 302 is preferably a snap-mounting mechanism. It is important that the coupling mechanism 310 provide a liquid-proof connection to ensure that any reagent in the probe 302 does not leak from the probe 302 in an uncontrolled manner during the transportation and dispensing of the reagent onto one or more of the slides.

The staining apparatus 303 comprises washing means 321, including two wash stations 304, 308, wherein the probes may be cleaned. The washing means 321 comprises at least two preferably identical wash stations 304, 308, a first wash station 304 and a second wash station 308. A lever arm 313 is provided, which is shiftable between the two wash stations 304, 308. The lever arm 313 moves a nozzle head 314 - or so-called hose turret - from the one wash station the other.

As shown in dotted lines in figure 301, dismounting means 311 are provided underneath a cover plate. The dismounting means 311 serves the purpose of deactivating the coupling mechanism 310 between the probe handling means 305 and the probe 302. With reference to Figure 302, the wash station and the dismounting means 311 are shown in a detailed view. In the embodiment shown, two wash stations are provided, a first wash station 304 and a second wash station 308. The wash stations 304, 308 are configured as elongate

cylinder units with a diameter that corresponds to at least the largest diameter of the probe and with a length that corresponds to the length of the probe 302.

The longitudinal axes of the cylinders are parallel with the longitudinal axis of the probe and are preferably vertical in orientation, whereby liquids are collected at the bottom of the wash station 304, 308 and from where it can be discharged. The apparatus 303 may comprise a number of wash stations. In a second embodiment of this aspect of the invention, the washing means includes a third wash station 329, which is essentially configured similar to the other wash stations 304, 308 (see figs. 308, 309a and 309b) for receiving the pipette tube section 330 of the probe 302. The wash station may also be able to comprise more than two probes that alternate between e.g. three and four as needed. The probes 302, 309 are reusable probes 302, 309, i.e. the probes used that are not disposable, but are instead being washed and subsequently reused in the same system/instrument as an integral part of the staining process.

In the open end of the first and second wash stations 304, 308 situated at the top, the probes 302, 309 will be inserted such that the mounting end 315 of the probe 302, 309, which is otherwise mounted on the robot arm 307 during transport and use, will be positioned at this top opening of the wash stations 304, 308. Besides, the wash station 304, 308 comprises a mechanism comprising a motor 326 with a pivot shaft 327 for pivoting the lever arm 313 between its positions. The lever arm 313 is mounted perpendicular to the shaft 327 and at the outermost end of said lever 313 a nozzle arrangement 314 is mounted. This nozzle head 314 is being moved between each washing operation from one wash station leaving a cleaned probe to another wash station where a used probe 302, 309 is delivered by the probe handling means 305. This nozzle 314 is provided with first and second connecting means 316, 317 in the form of a hose connection and in this case interconnected for influx of liquid and air. The nozzle head 314 is positioned and coupled to the top end of the probe 302, 309 and for subsequently rinsing the probe 302, 309, whereby the probe 302, 309 is washed along its outer periphery as well as within the tubular cavity of the probe. In this manner, it is ensured that the probe is absolutely clean following washing.

The coupling between the nozzle 314 and the probe 302, 309 may be any suitable coupling means. Accordingly, it could be a latch mechanism, e.g. a spring mechanism that may be released by mechanical means or by a pneumatic coupling, and wherein deactivation of this coupling means ensure that the coupling between nozzle 314 and probe 302 is deactivated.

In the embodiment shown the figures 301 and 302, a dismounting means 311 is used which is has the shape of two fork-structures, said dismounting means 311 structures are located adjacent each wash station 304, 308 and with the receiving forks in engagement opposite the coupling area between the nozzle 314 and the probe 302, 309. A second motor 328 activates the fork-structure 311 whereby it is shifted forwards and wherein the forks of the dismounting means 311 engages in the area between coupling and probe end face and accomplishes the deactivation of the coupling connection. When the process is finished, it is subsequently withdrawn.

This dismounting means 311 could also be designed in other ways, e.g. with a release mechanism including a pneumatic cylinder, whereby the deactivation can take place by releasing the pressure in the cylinder.

The nozzle head 314 is designed as a showerhead-type nozzle which is to be positioned directly above the open top of the end of the probe to be washed. The showerhead is provided with passages to direct fluid down into the interior of the probe 302, 309 and around the outside as well, thereby simultaneously cleaning both the interior and the exterior.

The showerhead may supply dry air as well as rinsing liquid and/or cleaning buffer. The showerhead turret will either rotate the head assembly in the horizontal plane.

When the probe 302 to be cleaned has been delivered in its wash station 304, the lever arm 313 will move the nozzle device 314 from the second wash station 308 to the first wash station, thereby exposing a clean probe 309 in the second wash station 308. A coupling

takes place between the probe 302 and the nozzle 314. The probe 302 will then subsequently be rinsed clean and blown clean.

Figure 303 shows an embodiment where the probe holder 307 in a position with the probe 302 mounted thereon and inserted in the first wash station 304. The dismounting means 311 for decoupling the probe from the probe handling means 305 is moved for detaching the probe, whereby the air cylinder connection or the mechanical spring connection between the two parts is deactivated.

Figure 304 shows the probe holding means 307 in a withdrawn position, wherein the probe holder 307 is moved upwards whereby the mounting means 310 are exposed. The nozzle head 314 is located in the second wash station 308. In Figure 305 the nozzle head 314 is moved from the second wash station 8 to the first wash station 304 by pivoting the lever arm 313. The nozzle head 314 and the free end of the delivered probe are coupled together, as is shown in Figure 305, and wherein a coupling to or an activation of the connecting means 316, 317 is subsequently performed to accomplish rinsing and drying of the probe interior and exterior.

The probe holding means 307 is redirected to the other wash station, where positioned and coupled to the clean second probe 309 situated in the second wash station 308.

As shown in Figure 307, the probe holder 307 is then withdrawn, and the second probe 309 is mounted, so that the orientation of the probe 309 is concentric with the axis of the probe holding means 7.

With reference to Figure 308, the wash station 321 is positioned essentially like the one shown in Figures 301-307. The robotic arm 305 moves the probe to a reagent vial section 319 of the apparatus. The probe 302 sucks up a predetermined amount of reagent liquid, which is necessary for treating the number of slides that are currently to be treated with the particular reagent. Additionally, the instrument will be required to perform a reagent inventory before a staining run can begin. This inventory is accomplished by use of the probe

tip to actually touch the fluid surface in each reagent bottle. This means that the probe will have to be washed after each reagent level is measured.

The arm is subsequently guided towards the slide section 320 of the apparatus which comprises a rack system in which a number of slides 306 are mounted. The slides 306 are mounted with a generally horizontal level and the probe releases the desired amount of reagent liquid on a selected slide according to a staining protocol.

Following the treatment of the slide or slides, the probe 302 is directed to the washing means 321, where the above-described washing process of the used probe takes place and where the probe on the probe handling means is replaced by a clean probe, whereby the process is repeated with a new reagent, said new reagent being selected according to the scheduled staining process.

Figs. 309a and 309b also shows a second embodiment of an apparatus 303 according to this aspect of the invention, wherein the reagent vial section 319 is arranged differently, and where there are three wash stations 304, 308 and 329, respectively, and a correspondingly designed nozzle head lever arm 313 for moving the nozzle 314 from the first to the second wash station. In this embodiment, the movement is a pendulum-like movement where the lever arm 313 with nozzle head 314 is undergoing a semi-circular run with two bottom positions.

In this embodiment, the third washings station 329 may accommodate a third probe, which is identical with the two others, but it may also be configured for mounting of a so-called manipulation stick (not shown). Said manipulation stick is mounted on the same probe handling means instead of a probe for exerting a mechanical manipulation on slides e.g. about an axis in those cases where the rack system is so configured. It may be necessary to contain this stick during the movements of the probe in order to avoid interference with other constructions of the apparatus. In the apparatus shown in Figure 9a, the slides are situated symmetrically around a first reagent vial section.

Figure 310 shows an embodiment of a design of a probe 302, 309, wherein this probe comprises an elongate tubular pipette unit 330 which is open at a first end 331 for sucking up reagent and for application. Opposite this end 315, an enlarged cylindrical portion 332 is provided that serves as reservoir. This cylindrical portion 332 comprises a collar 333 and above this collar 333, a cylindrical portion 334 with a diameter corresponding essentially to the cylindrical reservoir portion 332, but smaller than the collar portion 333. The collar 333 serves as coupling mechanism and sealing mechanism to the coupling means 310.

Thus, the probe comprises a lower pipette 330, a central reservoir 332 and an upper coupler 334. The probe 302, 309 is designed to penetrate a septum in the reagent bottle cap. The reagent sample is contained in the pipette and reservoir sections and does not contact the coupling portion. This prevents reagent contamination of the robot coupler and its components.

An embodiment of the coupling or latch mechanism 310 is shown Figure 311. A latch system is used to positively attach/detach the probes to/from the probe handling means at a wash station. This latch is used on both the pipette probe and slide manipulation probe. A probe is latched in place when a keyhold in a spring-loaded sliding plate engages a groove on the probe. O-rings provide the fluid seals. In the preferred embodiment, the coupling mechanisms 310 of the probe holding means 307 and nozzle head 314 are identical.

The latch actuator provides the motions and forces required to decouple the probes from the robot and the wash station. The actuator is a pivoted mechanism driven by a stepper motor through a series of linkages. Three "fingers" on the mechanism move to simultaneously depress the latches on all three probe locations; the two wash tubes for the pipette probes, and the storage tube for the slide manipulation probe. In each location, the actuator will depress both the lower latches, and the upper latch, which will be in one of the three tube locations. An optical sensor will monitor the position of the actuator.

Above, this aspect of the invention is described with reference to preferred embodiments of the invention. However, it is realised that variations and equivalent solutions

may be provided without departing from the scope of protection as defined in the accompanying claims.

As to the present aspect of the invention, some of the claims potentially to be presented and for which support should be understood to exist include:

1. A staining apparatus (303) for manipulation of samples arranged on carrier means (306), comprising probe means (302, 309) for dispensing a portion of reagent onto a selected carrier means (6), means (305) for handling said probe means (302, 309); and washing means (304, 308, 329) for cleaning the probe means (302, 309); characterised in that the probe means comprises at least two detachable probes (302, 309); and that the washing means includes at least two wash stations (304, 308, 329).

2. A staining apparatus according to claim 1, wherein the probe handling means (305) includes probe holding means (307) for holding a probe; and robot means for providing a relative movement between a selected probe (302, 309) and the washing means (304, 308, 329), including movement of the probe handling means (305) between the wash stations (306, 308).

3. A staining apparatus according to claim 2, wherein the probe handling means (305) includes dismounting means (311) for dismounting the probe (302, 309) from the probe holding means (307).

4. A staining apparatus according to claim 2 or 3, wherein the probe handling means (305) includes coupling means (310) for providing a detachable, fluid sealed coupling between the probe (302, 309) and the probe holding means (307).

5. A staining apparatus according to claim 4, wherein said coupling means (310) includes probe coupling portion (312) on the probe holding means (307),

which engages a corresponding receiving portion (315) on the probe (302, 309) and latching means for releasably attaching the probe (302, 309) to the probe handling means (305).

6. A staining apparatus according to claim 4 or 5, wherein the coupling means (310) comprises an air cylinder for releasing the latching means and allow decoupling of the probe (302, 309).

7. A staining apparatus according to any of the preceding claims, wherein the washing means include a first wash station (304) and the second wash station (308), said wash stations (304, 308) being identical.

8. A staining apparatus according to any of the preceding claims, wherein the washing means comprises a nozzle head (314), which is mounted on a lever (313), which is movable between the wash stations (304, 308) by shifting means (326, 327).

9. A staining apparatus according to claim 8, wherein the nozzle head (314) comprises a showerhead nozzle, said nozzle being arranged to engage the receiving portion (315) of the probe (302, 309) to be washed.

10. A staining apparatus according to claim 8 or 9, wherein the nozzle head (314) comprises an air supply means (316).

11. A staining apparatus according to any of claims 8 to 10, wherein the nozzle head (314) comprises a cleaning buffer supply means (317).

12. A staining apparatus according to any of claims 8 to 11, wherein the nozzle head (314) distributes cleaning fluid to the interior and the exterior of the probe (302, 309).

13. A method of washing probes in a staining apparatus (303) for manipulation of samples arranged on carrier means (306) including a first probe (302, 309) for dispensing a portion of reagent onto a selected carrier means (306), means (305) for handling said first probe (302), and washing means (304, 308, 329) for cleaning the probe (302); said method involving the following steps:

delivering the first probe (302) to a wash station (304) of the washing means after dispensing reagent onto the carrier means (308); and
detaching said probe (302) from the probe handling means (305) in said wash station (304, 308).

14. A method according to claim 13, whereby the probe handling means (305) are moved away from said first wash station (304) for selecting a clean second probe (309) in a second wash station (308).

15. A method according to claim 13 or 14, whereby a nozzle cleaning head (314) is moved to the first wash station (304) and engages the first probe (302) subsequent to its delivery in said wash station (304) for washing said first probe (302).

16. A method according to claim 15, whereby the probe (302) is rinsed with cleaning fluid supplied through the nozzle head (314).

17. A method according to claim 15 or 16, whereby air supplied through the nozzle head (314) is blown through the probe (302).

18. A method according to any of claims 15 to 17, whereby a buffer solution is applied through the nozzle head (314) to the probe (302).

19. A method according to claim 13 to 18, whereby the nozzle head (314) is shifted between the first and second wash stations (304, 308) for successive probe washing.

20. A method according to claim 14, whereby a second probe (309) is coupled to the probe handling means (305) and moved away from the washing means for being prepared for dispensing a reagent by collecting a reagent from a predetermined reagent supply and dispensing this portion of reagent onto a predetermined carrier means (308).

In brief, this aspect of the invention may be understood as involving, among other aspects, a staining apparatus for manipulation of samples arranged on carrier means (306) and a method of washing probes. Said apparatus comprising probe means (302, 309) for dispensing a portion of reagent onto a selected carrier means (306), means (305) for handling said probe means (302, 309); and washing means (304, 308, 329) for cleaning the probe means (302, 309); wherein the probe means comprises at least two detachable probes (2, 9); and that the washing means includes at least two wash stations (304, 308, 329). According to this aspect of the invention, the probe handling means may release the first probe at one wash station and quickly pick-up a clean probe for the next reagent dispensing run. Hereby, the probe washing and the reagent dispensing processes may take place simultaneously rather than sequentially, as it is the case in the prior art. This results in a significant reduction of time for the total process run, as the delay in reagent dispensing is kept to a minimum.

As can be easily understood from the foregoing, the basic concepts of the present invention may be embodied in a variety of ways. It involves both sample processing techniques as well as various systems, assemblies, and devices to accomplish sample processing and other functions. In this application, the sample processing techniques are also disclosed as part of the results shown to be achieved by the various systems, assemblies, and devices described and as steps which are inherent to utilization. They should be understood to be the natural result of utilizing the devices as intended and described. In addition, while some devices are disclosed, it should be understood that these not only accomplish certain

methods but also can be varied in a number of ways. Importantly, as to all of the foregoing, all of these facets should be understood to be encompassed by this disclosure.

The discussion included in this provisional application is intended to serve as a basic description. The reader should be aware that the specific discussion may not explicitly describe all embodiments possible; many alternatives are implicit. It also may not fully explain the generic nature of the invention and may not explicitly show how each feature or element can actually be representative of a broader function or of a great variety of alternative or equivalent elements. Again, these are implicitly included in this disclosure. Where the invention is described in device-oriented terminology, each element of the device implicitly performs a function. Apparatus claims may not only be included for the device described, but also method or process claims may be included to address the functions the invention and each element performs. Neither the description nor the terminology is intended to limit the scope of the claims which will be included in a full patent application.

It should also be understood that a variety of changes may be made without departing from the essence of the invention. Such changes are also implicitly included in the description. They still fall within the scope of this invention. A broad disclosure encompassing both the explicit embodiment(s) shown, the great variety of implicit alternative embodiments, and the broad methods or processes and the like are encompassed by this disclosure and may be relied upon when drafting the claims for presentation in any full or subsequent patent application. It should be understood that such language changes and broad claiming will be accomplished when the applicant later (filed by the required deadline) seeks a patent filing based on this provisional filing. The subsequently filed, full patent application will seek examination of as broad a base of claims as deemed within the applicant's right and will be designed to yield a patent covering numerous aspects of the invention both independently and as an overall system.

Further, each of the various elements of the invention and claims may also be achieved in a variety of manners. This disclosure should be understood to encompass each such variation, be it a variation of an embodiment of any apparatus embodiment, a method or

process embodiment, or even merely a variation of any element of these. Particularly, it should be understood that as the disclosure relates to elements of the invention, the words for each element may be expressed by equivalent apparatus terms or method terms -- even if only the function or result is the same. Such equivalent, broader, or even more generic terms should be considered to be encompassed in the description of each element or action. Such terms can be substituted where desired to make explicit the implicitly broad coverage to which this invention is entitled. As but one example, it should be understood that all actions may be expressed as a means for taking that action or as an element which causes that action. Similarly, each physical element disclosed should be understood to encompass a disclosure of the action which that physical element facilitates. Regarding this last aspect, as but one example, the disclosure of a "retention element" should be understood to encompass disclosure of the act of "retaining" -- whether explicitly discussed or not -- and, conversely, were there effectively disclosure of the act of "retaining", such a disclosure should be understood to encompass disclosure of a "retention element" and even a "means for retaining". It should also be understood that in jurisdictions where specific language may be construed as limiting, as but one example in the United States where some interpretations of "means for" elements can be construed narrowly (such as in the terms "" or the like), broader equivalent language (such as " element" or the like) may be used and should be understood as encompassed by this specification. Such changes and alternative terms are to be understood to be explicitly included in the description.

Any acts of patents, patent applications, publications, or other references mentioned in this application for patent are hereby incorporated by reference. In addition, as to each term used it should be understood that unless its utilization in this application is inconsistent with such interpretation, common dictionary definitions should be understood as incorporated for each term and all definitions, alternative terms, and synonyms such as contained in the Random House Webster's Unabridged Dictionary, second edition are hereby incorporated by reference. Finally, all references listed in the attached list of References To Be Incorporated By Reference In Accordance With The Provisional Patent Application filed with the application are hereby appended and hereby incorporated by reference, however, as to each of the above, to the extent that such information or statements incorporated by

reference might be considered inconsistent with the patenting of this/these invention(s) such statements are expressly not to be considered as made by the applicant(s).

Thus, the applicant(s) should be understood to claim at least: i) each of the sample processing systems and subsystems as herein disclosed and described, ii) the related methods disclosed and described, iii) similar, equivalent, and even implicit variations of each of these systems, assemblies, devices and methods, iv) those alternative designs which accomplish each of the functions shown as are disclosed and described, v) those alternative designs and methods which accomplish each of the functions shown as are implicit to accomplish that which is disclosed and described, vi) each feature, component, and step shown as separate and independent inventions, vii) the applications enhanced by the various systems or components disclosed, viii) the resulting products produced by such systems or components, and ix) methods and systems, assemblies, devices, and apparatuses substantially as described hereinbefore and with reference to any of the accompanying examples, x) the various combinations and permutations of each of the elements disclosed, xi) each potentially dependent claim or concept as a dependency on each and every one of the independent claims or concepts presented, xii) processes performed with the aid of or on a computer as described throughout the above discussion, xiii) a programmable system as described throughout the above discussion, xiv) a computer readable memory encoded with data to direct a computer comprising means or elements which function as described throughout the above discussion, xv) a computer configured as herein disclosed and described, xvi) individual or combined subroutines and programs as herein disclosed and described, xvii) the related methods disclosed and described, xviii) similar, equivalent, and even implicit variations of each of these systems and methods, xix) those alternative designs which accomplish each of the functions shown as are disclosed and described, xx) those alternative designs and methods which accomplish each of the functions shown as are implicit to accomplish that which is disclosed and described, xxi) each feature, component, and step shown as separate and independent inventions, and xxii) the various combinations and permutations of each of the above.

Further, if or when used, the use of the transitional phrase "comprising" or the like is used to maintain the "open-end" claims herein, according to traditional claim interpretation. Thus, unless the context requires otherwise, it should be understood that the term "comprise" or variations such as "comprises" or "comprising" or the like, are intended to imply the inclusion of a stated element or step or group of elements or steps but not the exclusion of any other element or step or group of elements or steps. Such terms should be interpreted in their most expansive form so as to afford the applicant the broadest coverage legally permissible.

Any claims set forth at any time are hereby incorporated by reference as part of this description of the invention, and the applicant expressly reserves the right to use all of or a portion of such incorporated content of such claims as additional description to support any of or all of the claims or any element or component thereof, and the applicant further expressly reserves the right to move any portion of or all of the incorporated content of such claims or any element or component thereof from the description into the claims or vice-versa as necessary to define the matter for which protection is sought by this application or by any subsequent continuation, division, or continuation-in-part application thereof, or to obtain any benefit of, reduction in fees pursuant to, or to comply with the patent laws, rules, or regulations of any country or treaty, and such content incorporated by reference shall survive during the entire pendency of this application including any subsequent continuation, division, or continuation-in-part application thereof or any reissue or extension thereon.

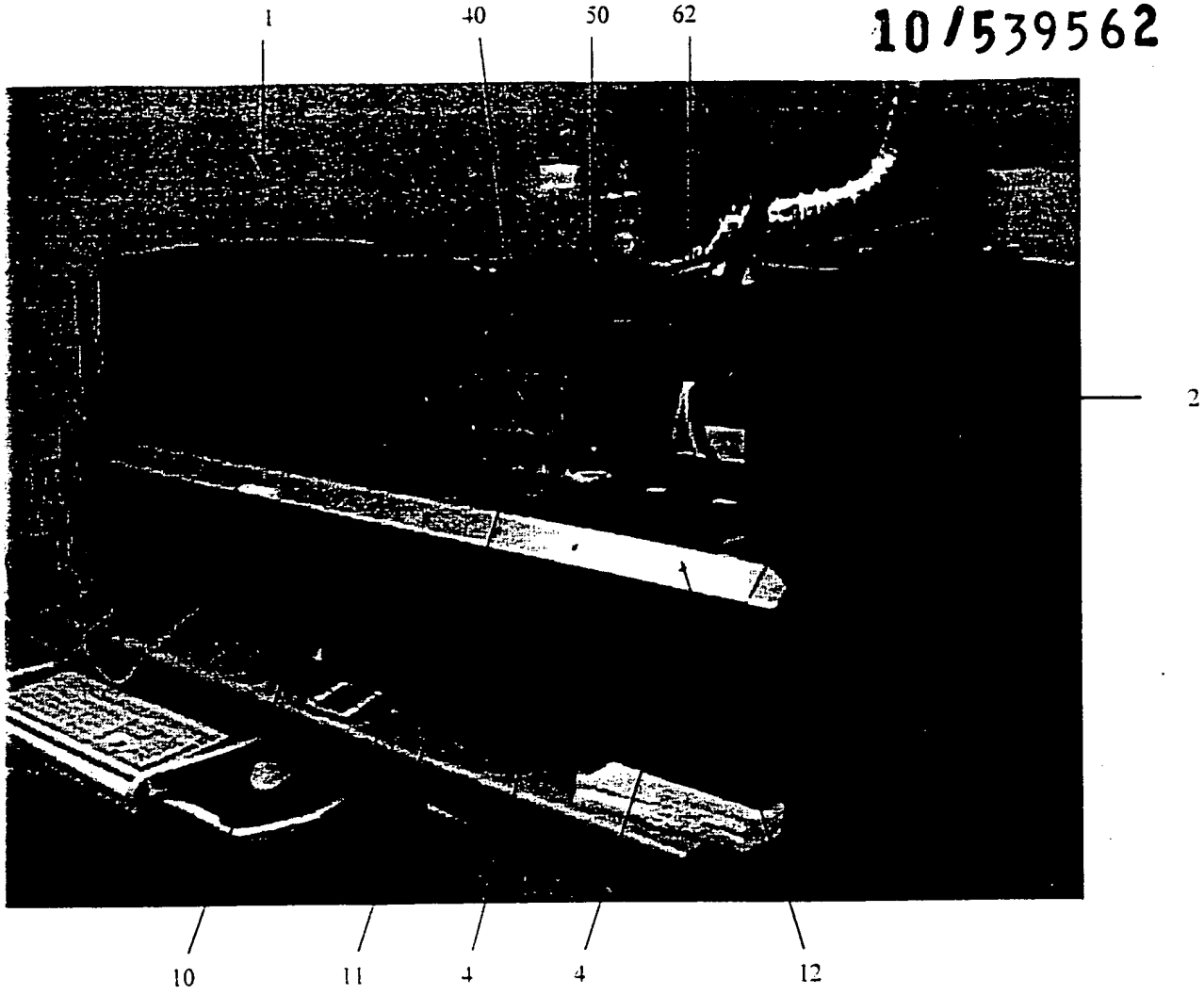

Figure A

Figure B

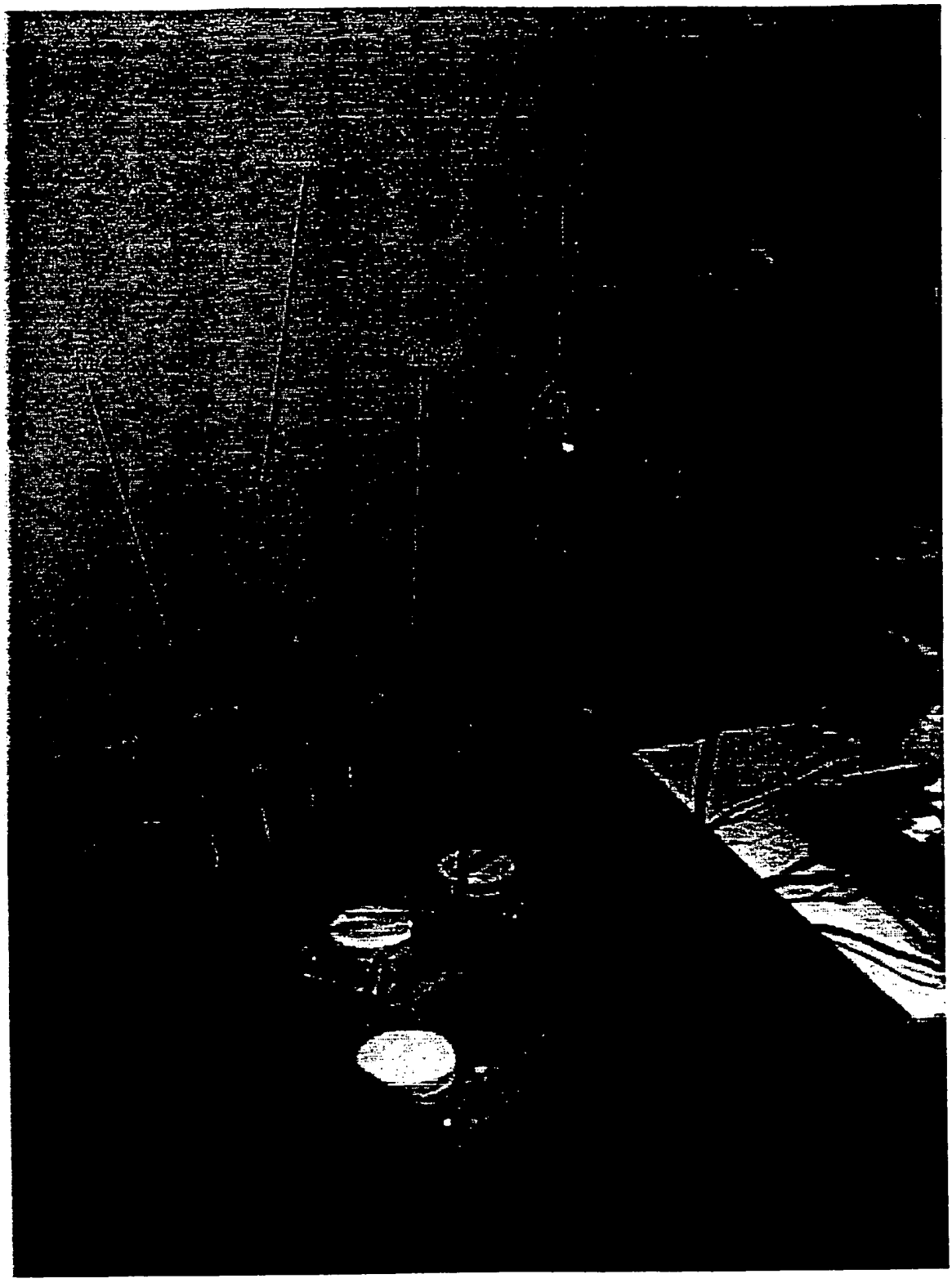

Figure C

Figure D

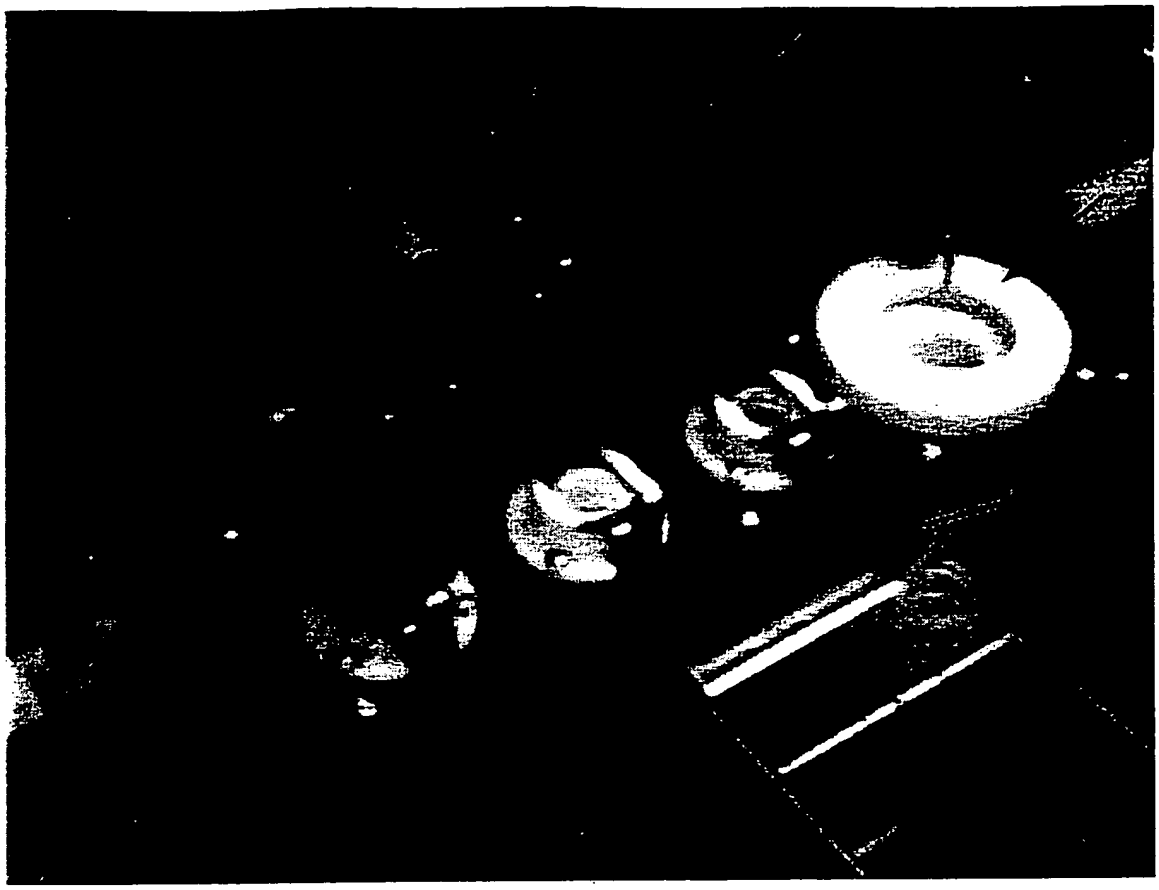

Figure E

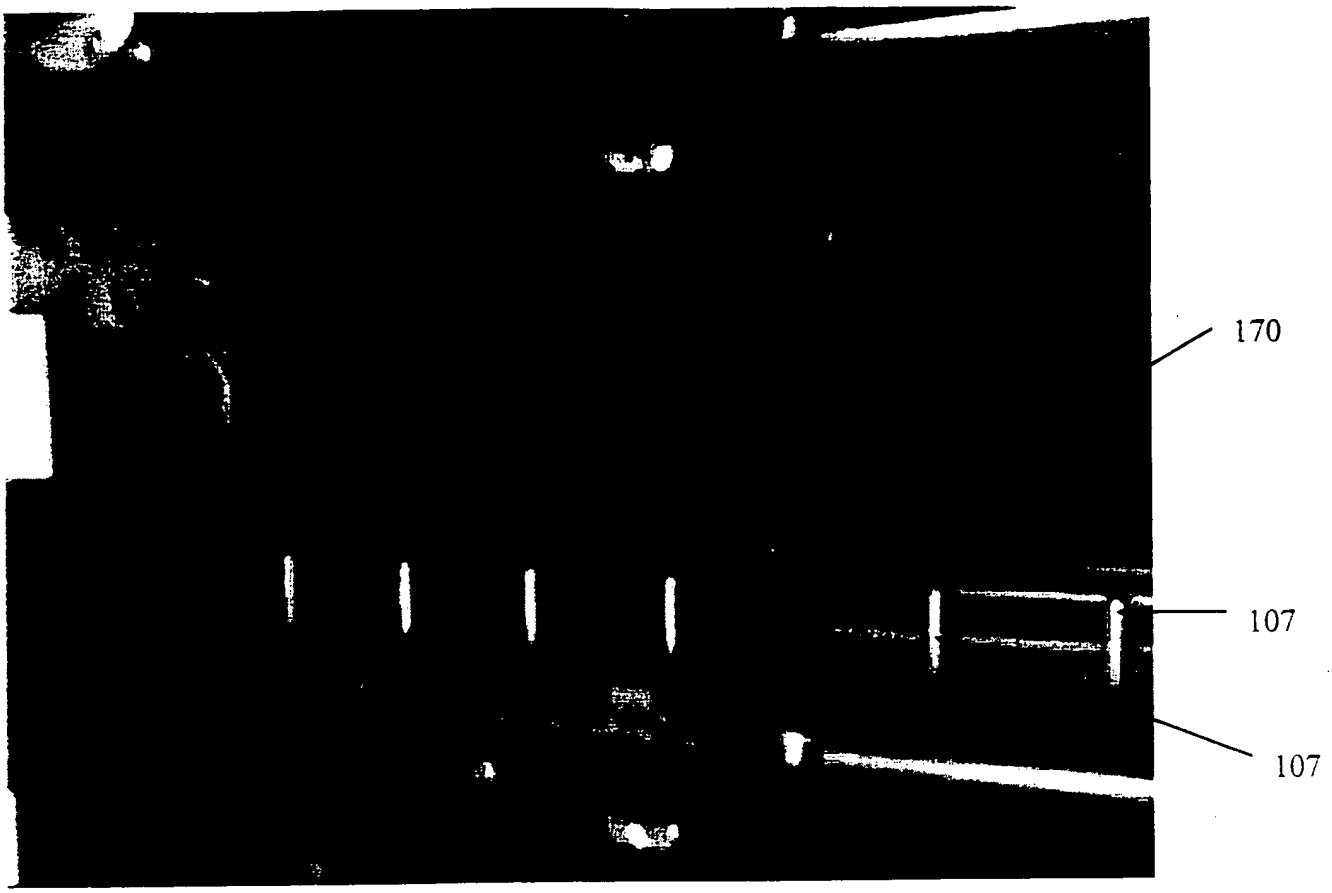

Figure F

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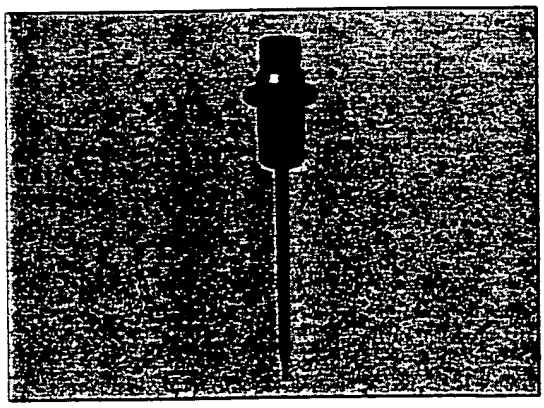
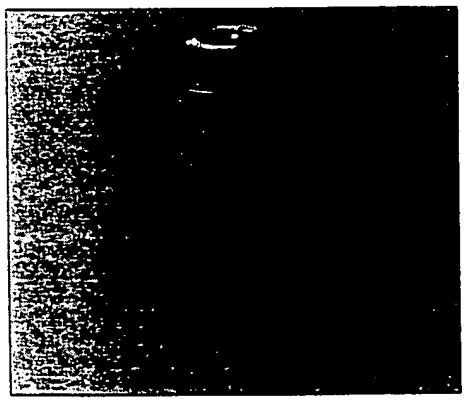
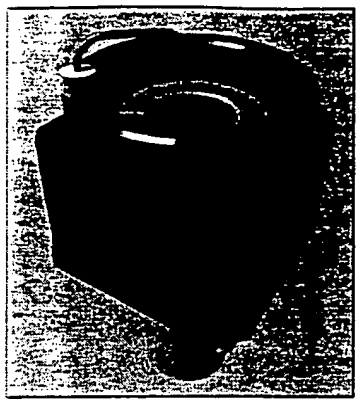


101b

101c



Figures 101



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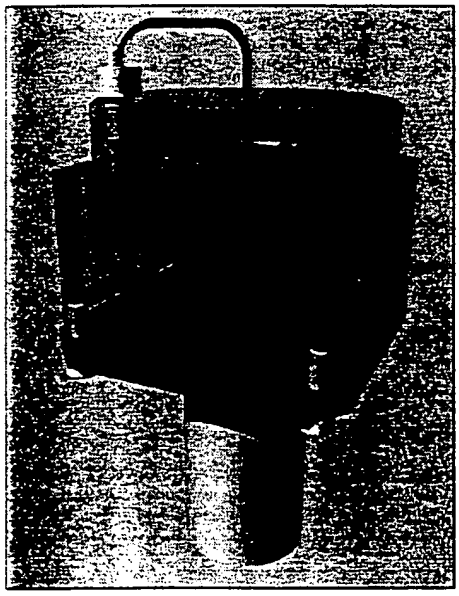
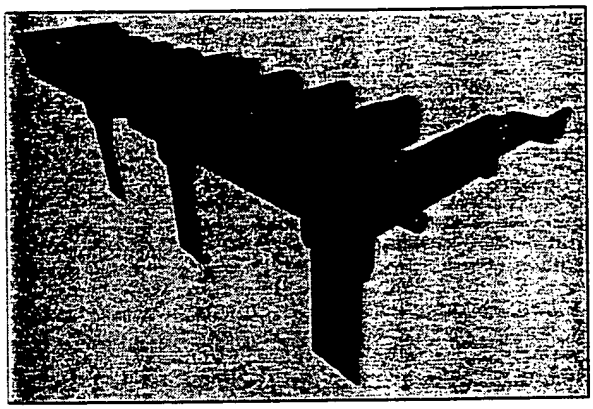
102a



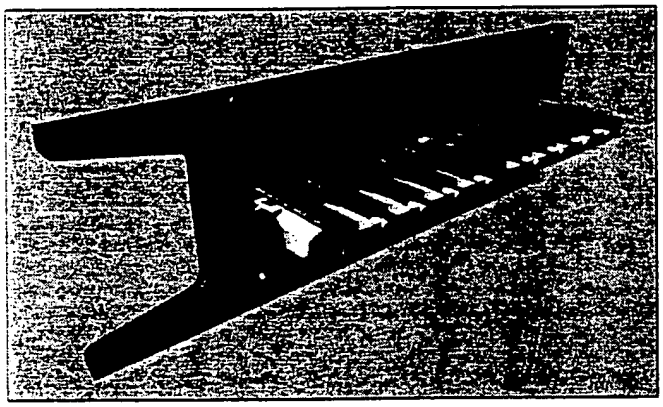
102b

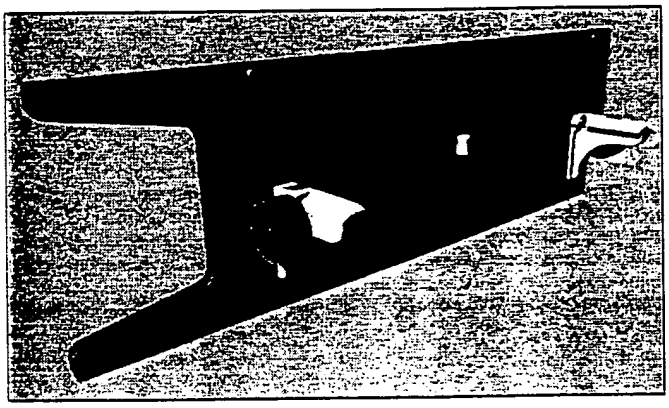
Figures 102

103a

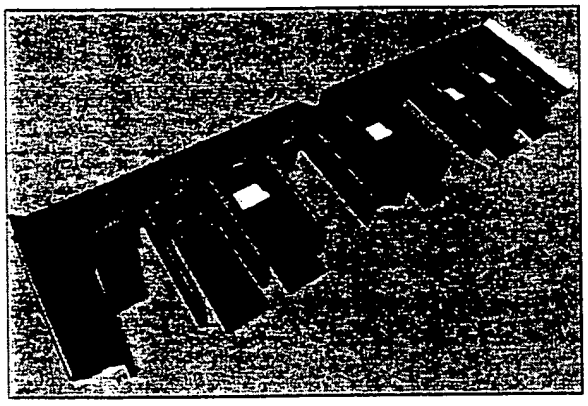


103b





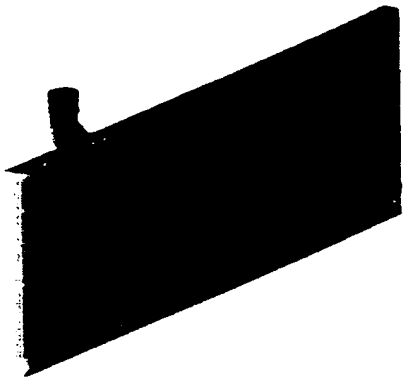
103c

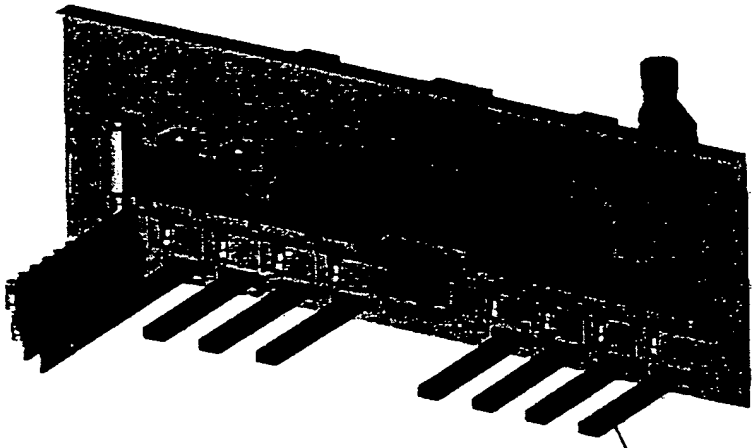


103d

Figures 103

104a



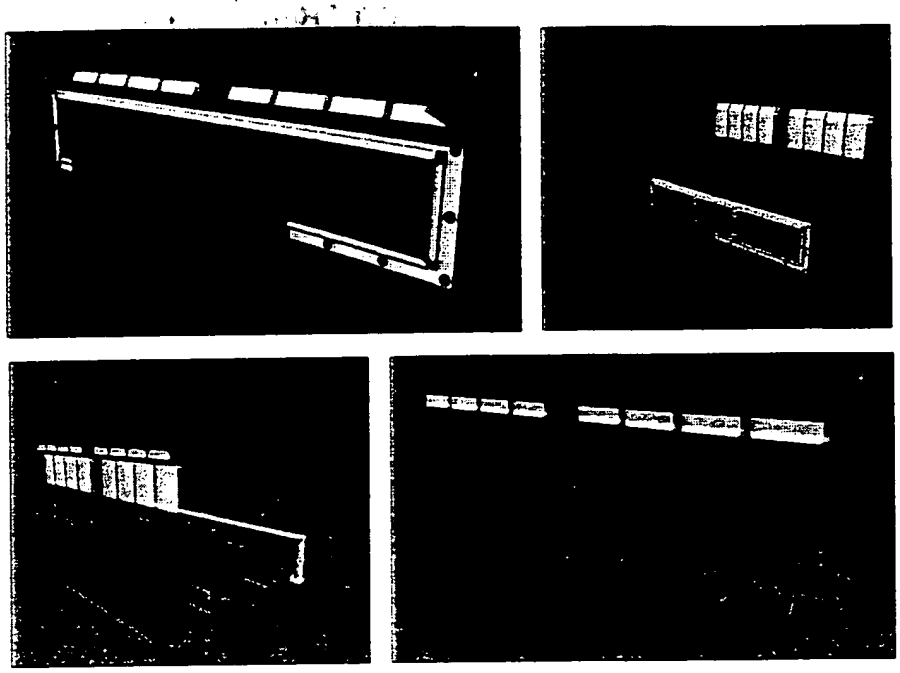


Figures 104

105a

105b

70



105c

105d

Figures 105

106a

106b

80

106c

Figures 106

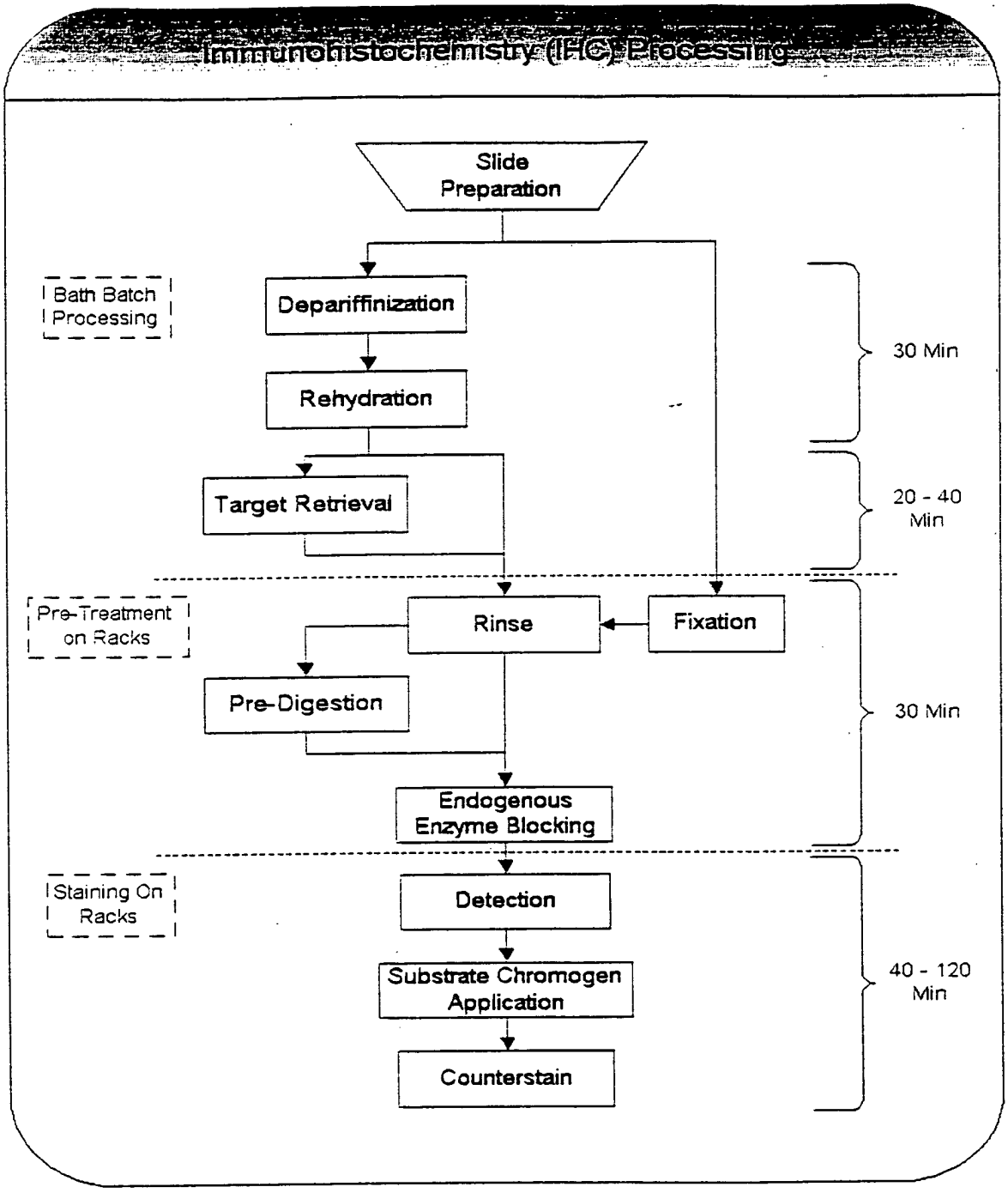

Immunohistochemistry (IHC) Processing
Slide Preparation
Bath Batch Processing
Depariffinization
Rehydration
30 Min
Target Retrieval
20 - 40 Min
Pre-Treatment on Racks
Rinse
Fixation
Pre-Digestion
30 Min
Endogenous Enzyme Blocking
Staining On Racks
Detection
Substrate Chromogen Application
Counterstain
40 - 120 Min

Figure 107

IHC Deparaffinization Process:

Process	Protocol Step	Time (min)	Temp C	Waste Segregation
Deparaffinization	Switch			Hazardous Waste
	Histoclear	5		
	Drain			
	Histoclear	5		
	Drain			
Re-Hydration	100% Ethanol	5		
	Drain			
	100% Ethanol	5		
	Drain			
	95% Ethanol	5		
	Drain			
	95% Ethanol	5		
	Rinse - Water	5		
	Switch			Non-Hazardous Waste
Target Retrieval	Target Retrieval	20	95	
	Target Retrieval Cool	20	55	
	Rinse - Water	5	RT	
Enzyme/Antibody Application	Peroxide Block	5		
	Enzyme Pretreatment	5		
	Rinse - Buffer			
	Pre-Diluted Antibody	10		
	Rinse - Buffer			
	EnVision-HRP	10		
Chromogen/ Counterstain Treatment	Rinse - Buffer			
	Switch			Hazardous Waste
	DAB	5		
	Rinse - Buffer			
	Hematoxylin	5		
	Rinse - Water			

Figure 108

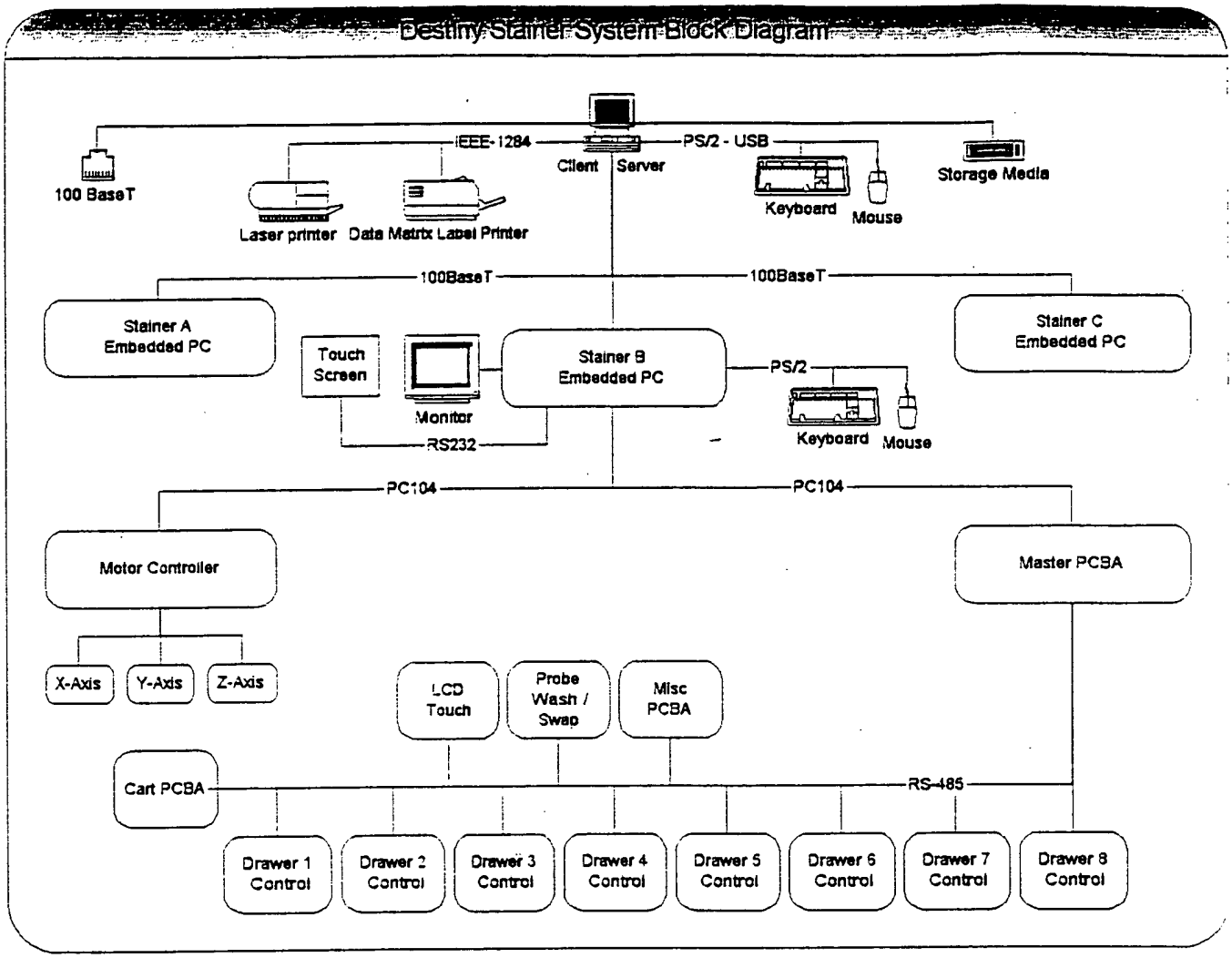

Destiny Stainer System Block Diagram
100 BaseT
IEEE-1284
Client
Server
PS/2 - USB
Storage Media
Laser printer
Data Matrix Label Printer
Keyboard
Mouse
100BaseT
100BaseT
Stainer A
Embedded PC
Touch
Screen
Monitor
Stainer B
Embedded PC
PS/2
Stainer C
Embedded PC
RS232
Keyboard
Mouse
PC104
PC104
Motor Controller
Master PCBA
X-Axis
Y-Axis
Z-Axis
LCD
Touch
Probe
Wash /
Swap
Misc
PCBA
Cart PCBA
RS-485
Drawer 1
Control
Drawer 2
Control
Drawer 3
Control
Drawer 4
Control
Drawer 5
Control
Drawer 6
Control
Drawer 7
Control
Drawer 8
Control

Figure 109

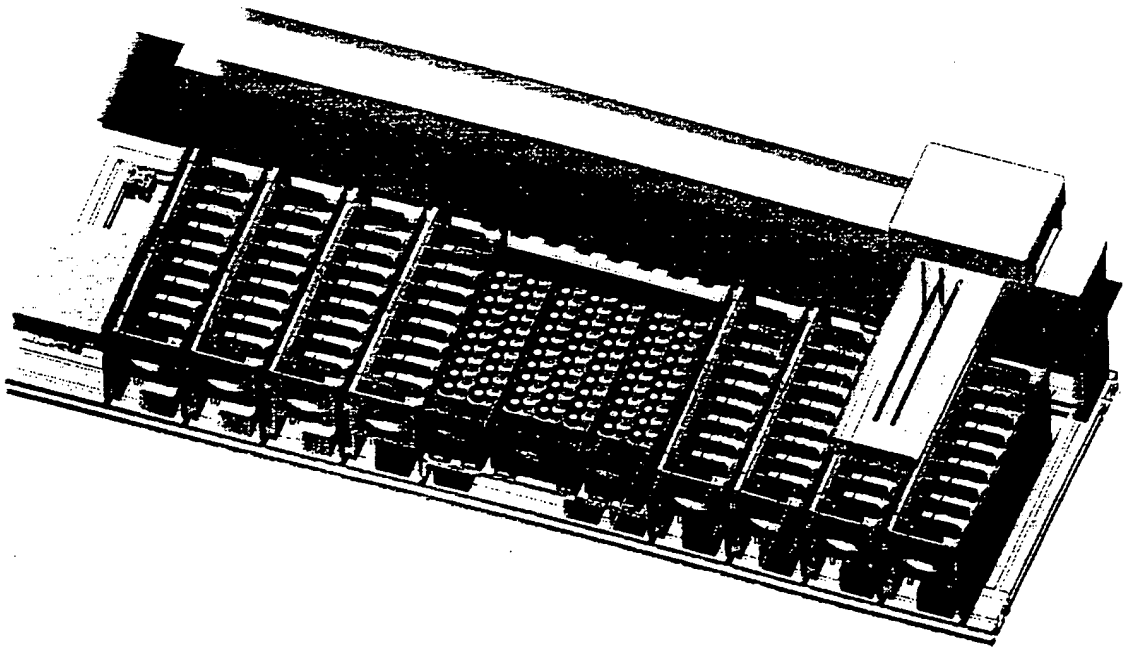


Figure 110

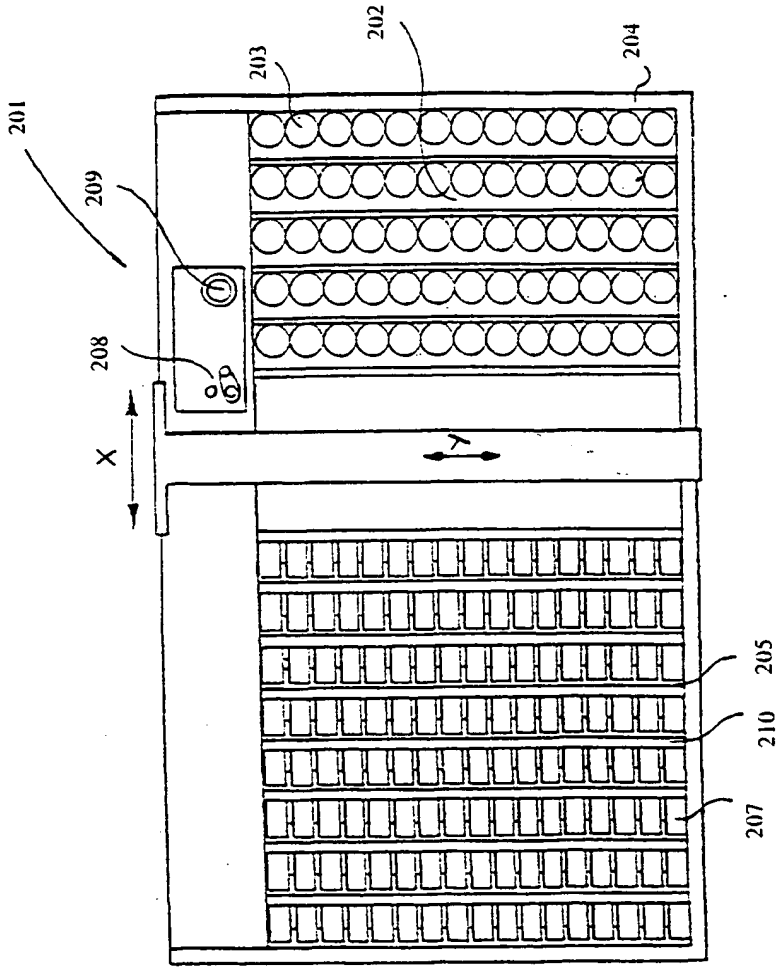


Figure 201

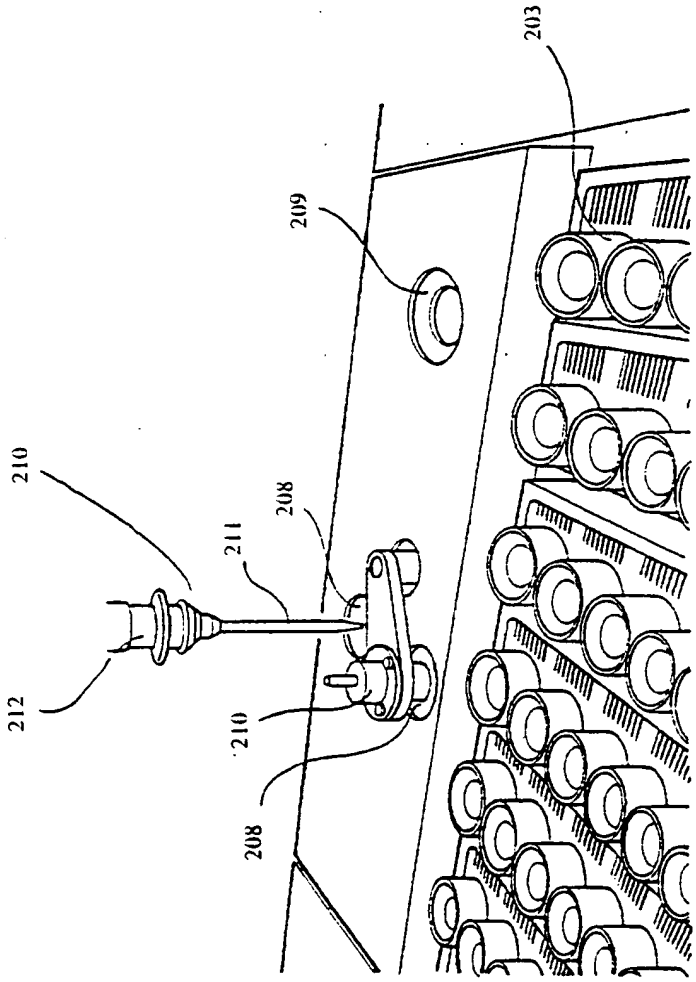


Figure 202

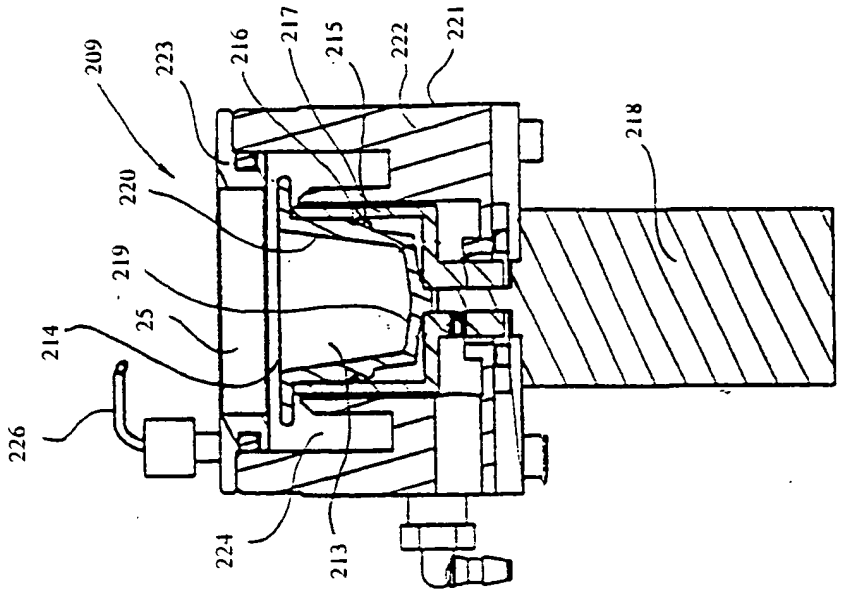


Figure 204

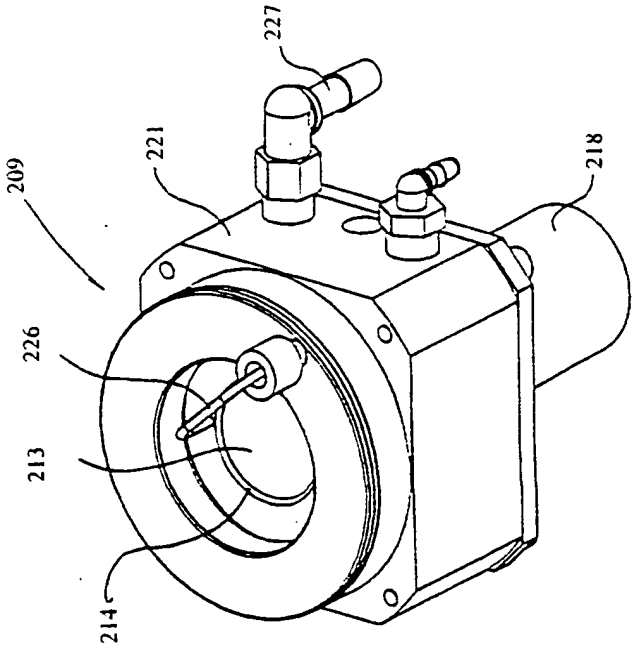


Figure 203

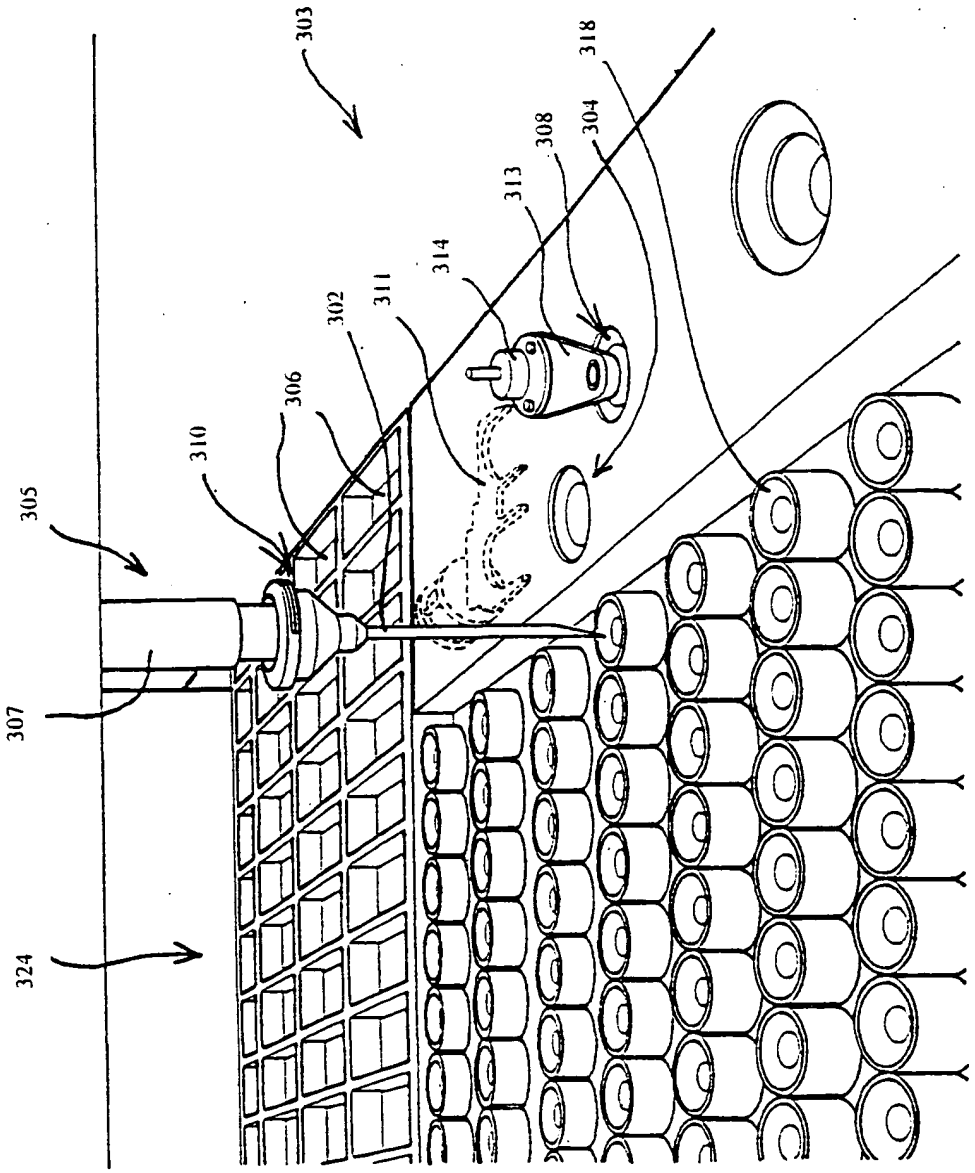


Figure 301

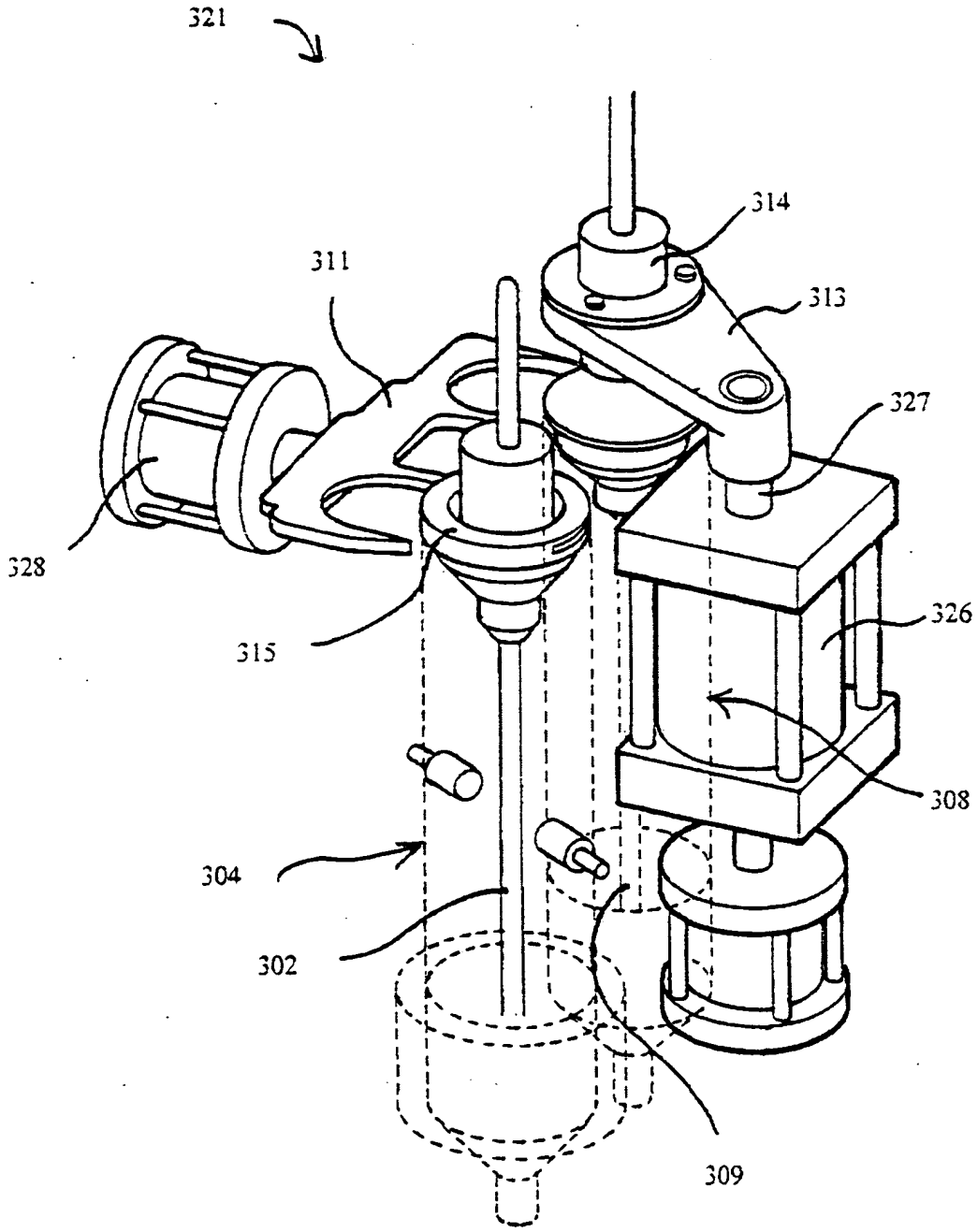


Figure 302

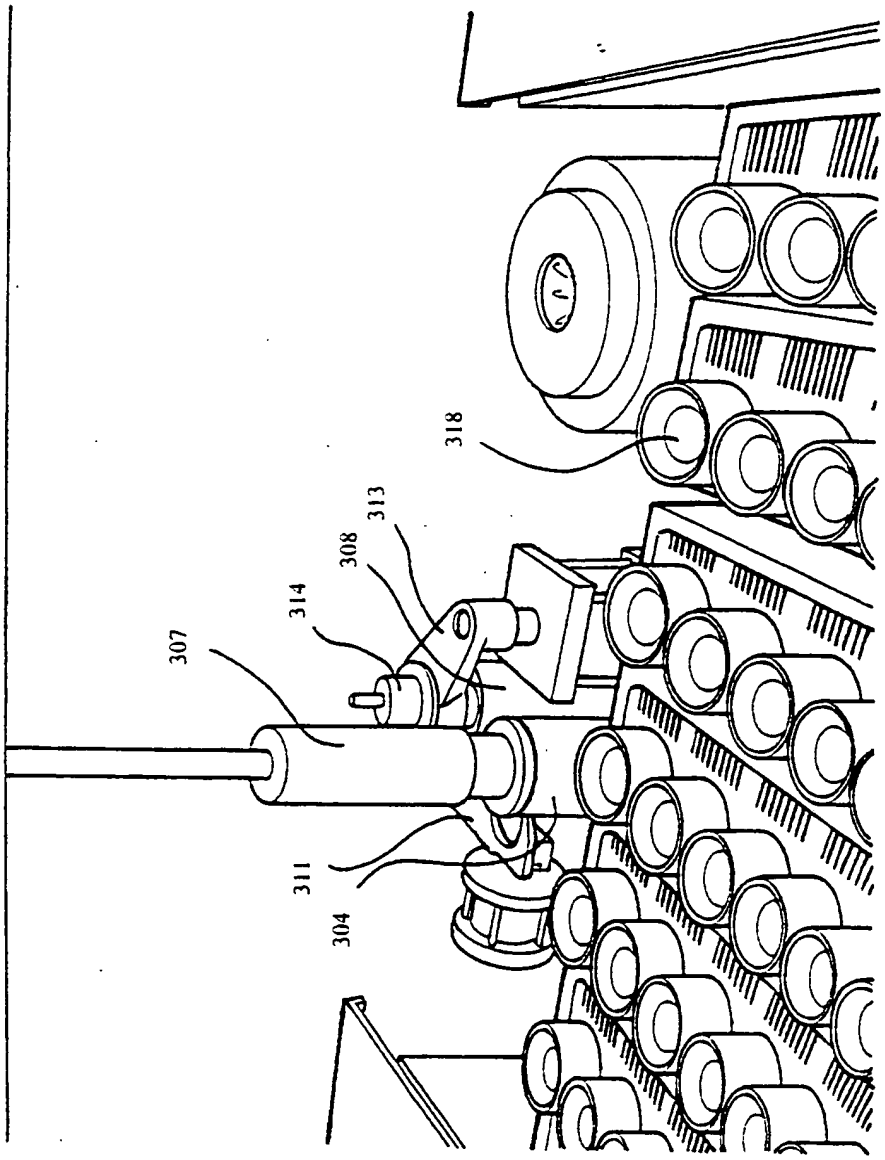


Figure 303

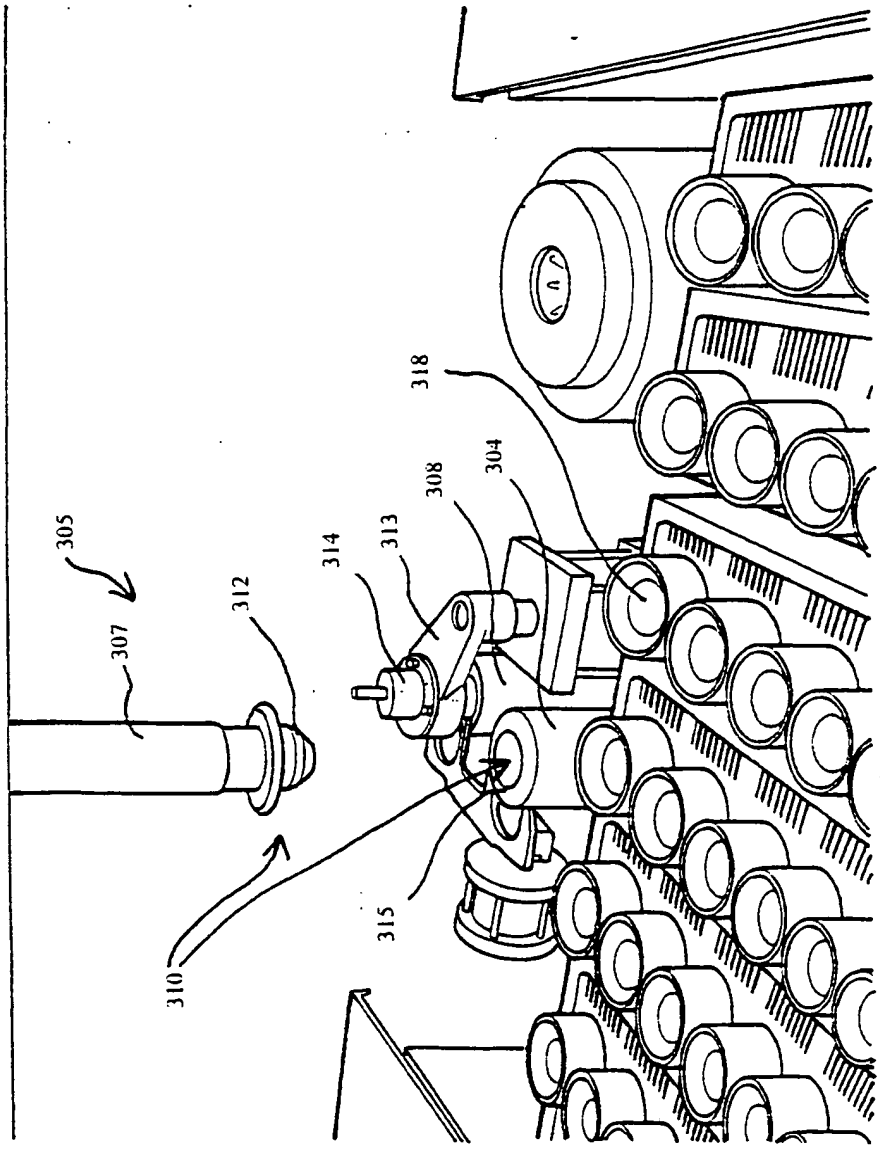


Figure 304

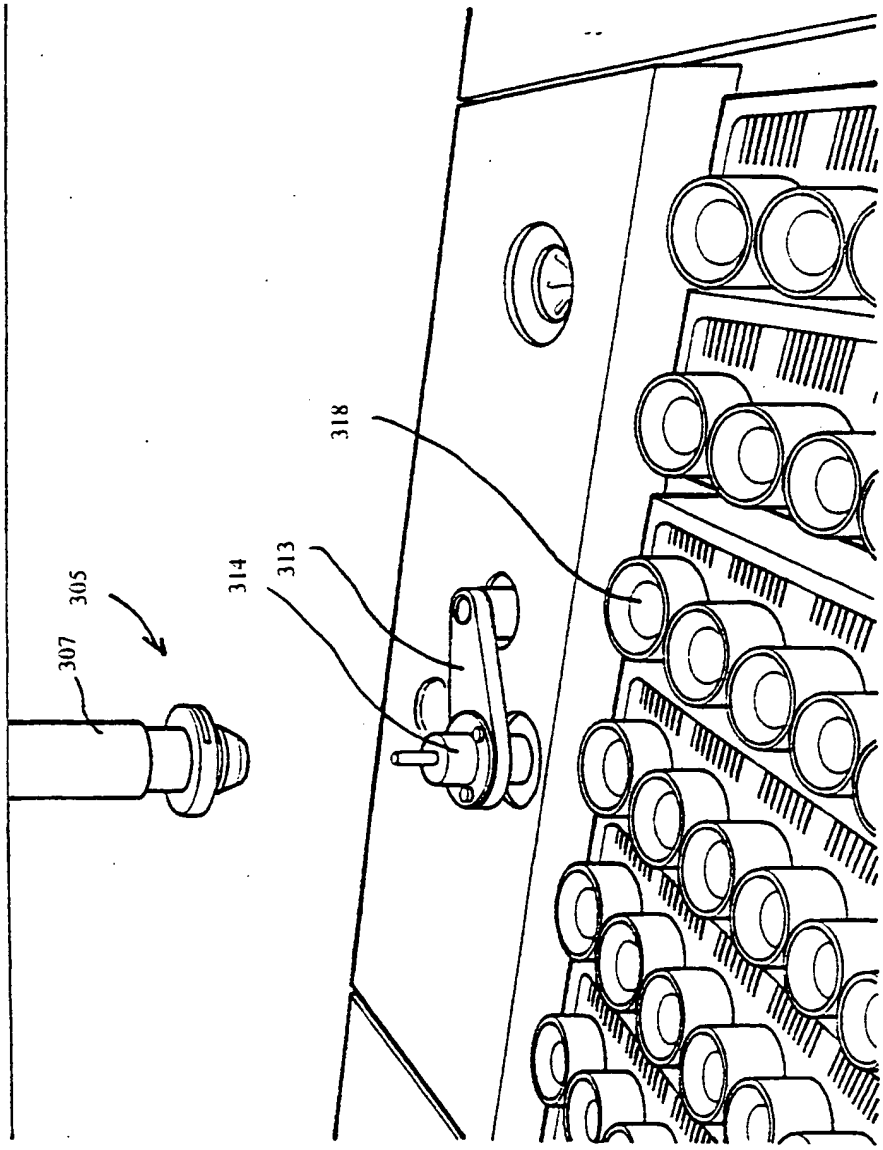


Figure 305

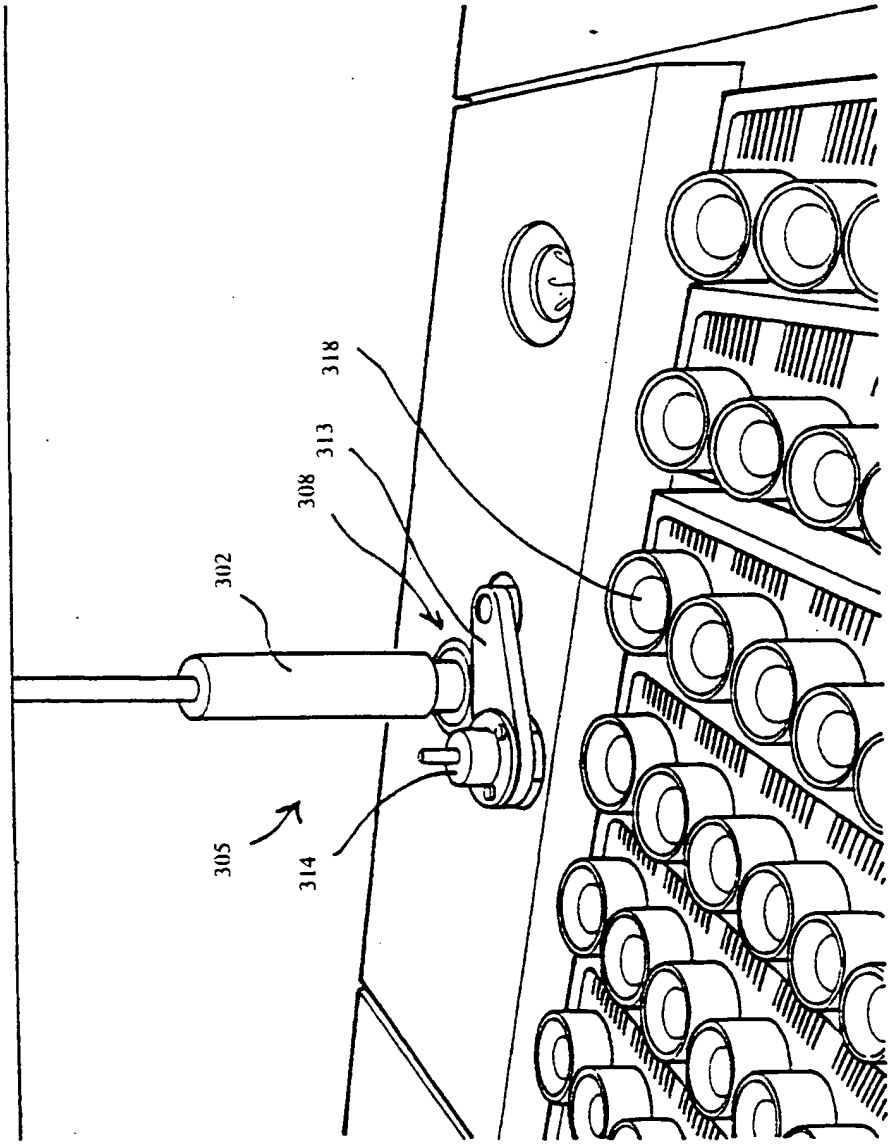


Figure 306

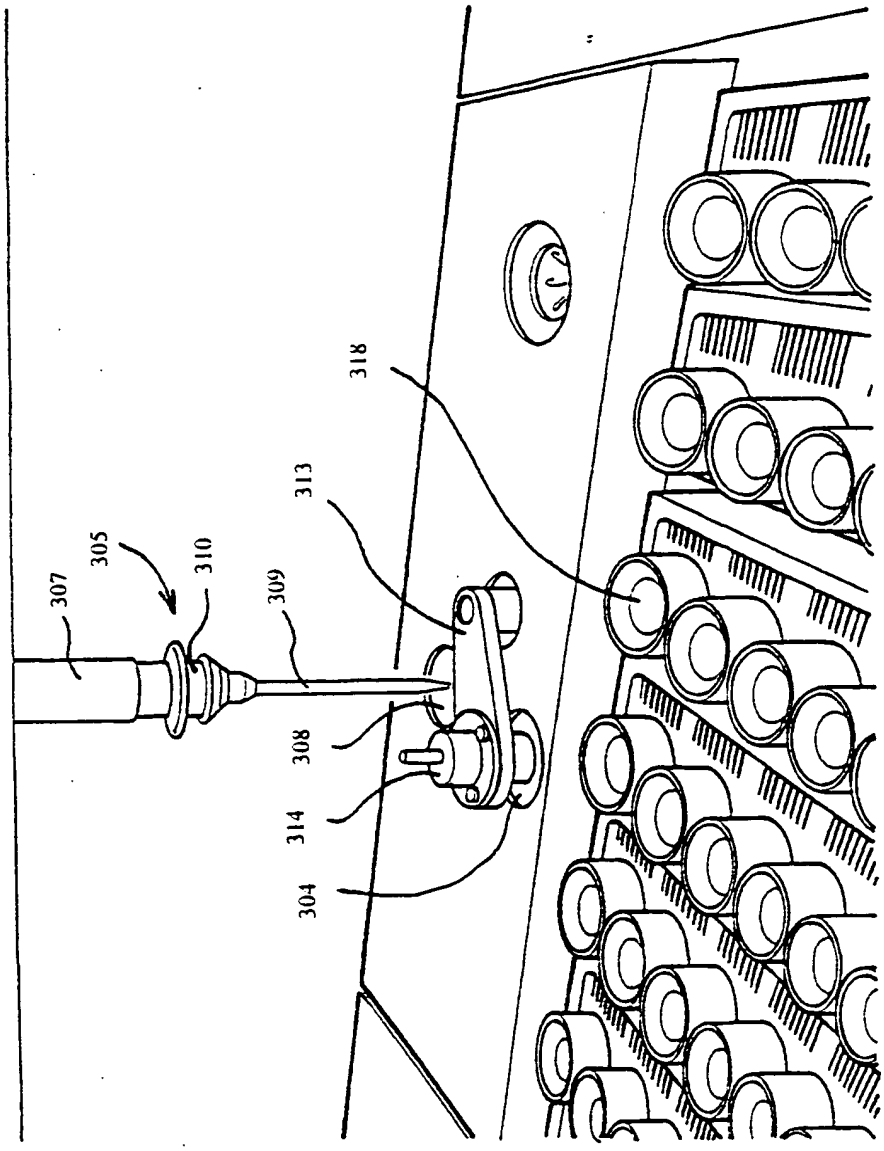


Figure 307

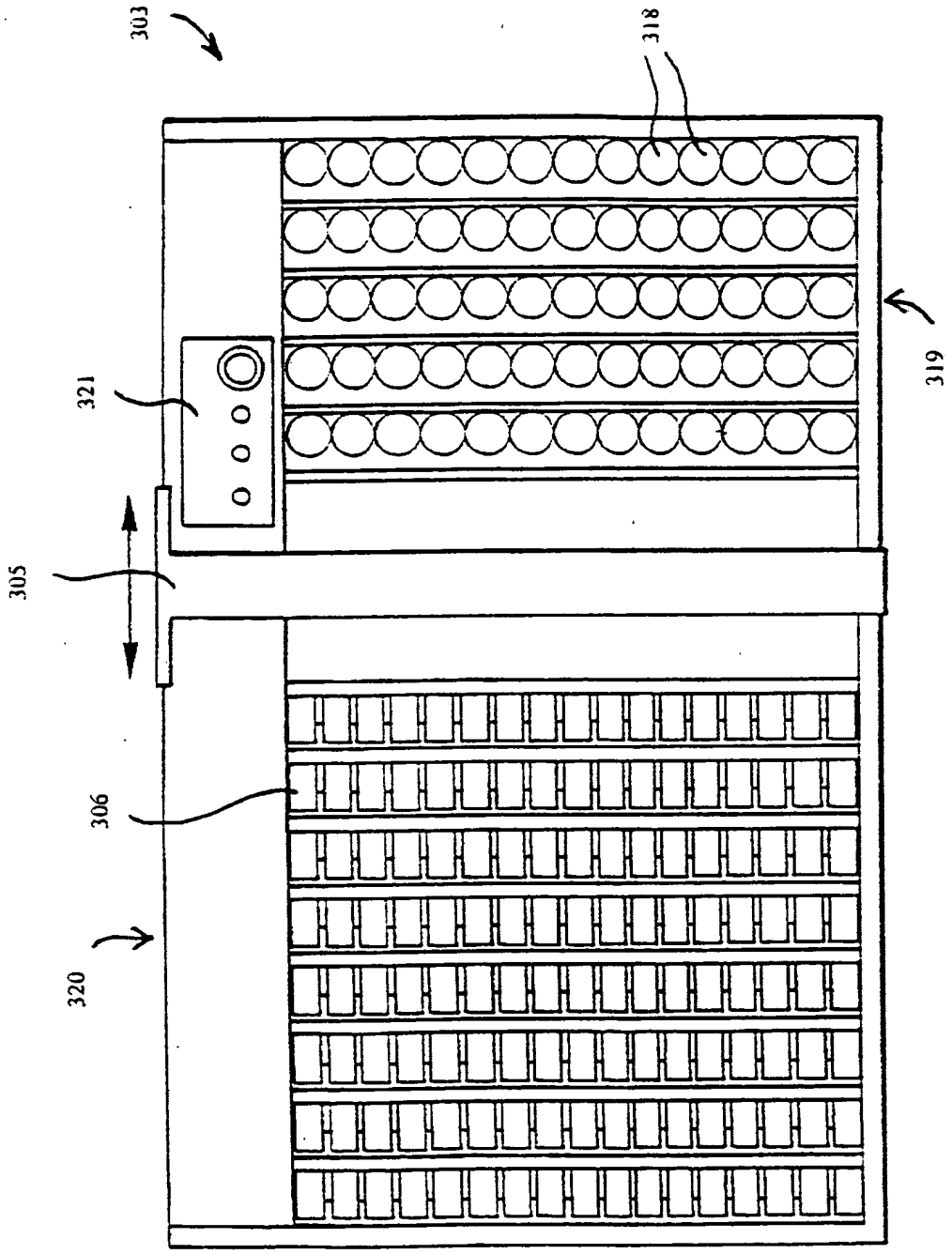


Figure 308

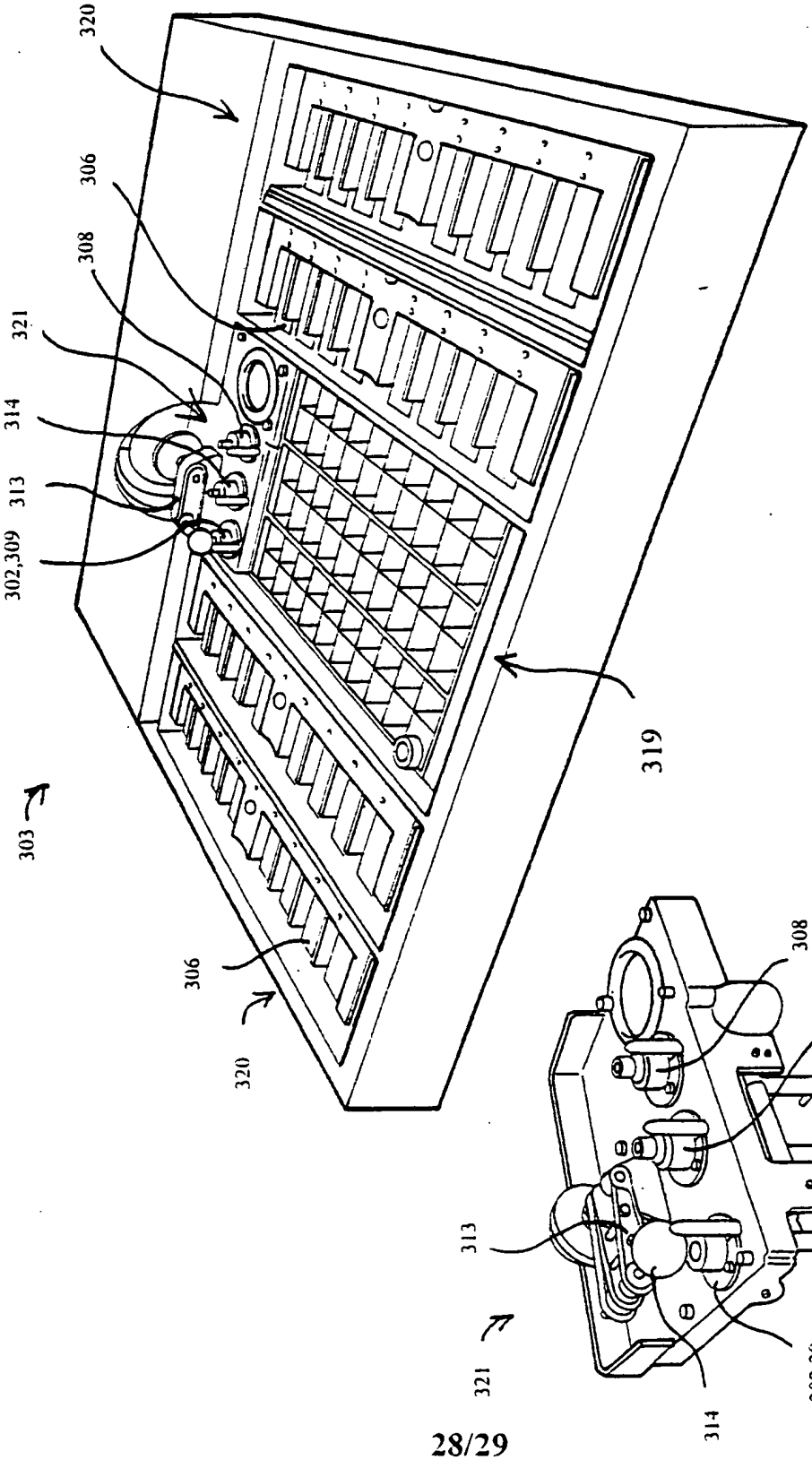
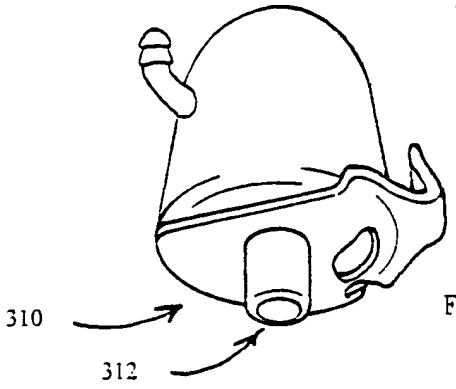


Figure 309A

Fig.ure 309B



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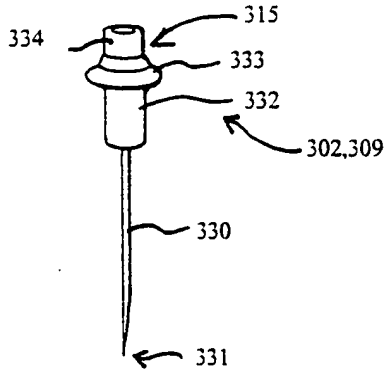

Figure 310